1-1-2004

Cooling meat products in foodservice: time, temperature, and growth of Clostridium perfringens ATCC 10388

David A. Olds
Iowa State University

Cooling meat products in foodservice: Time, temperature, and growth of *Clostridium perfringens* ATCC 10388

by

David A. Olds

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Foodservice and Lodging Management

Program of Study Committee:
Jeannie Sneed, Major Professor
Aubrey Mendonca
Amit Sharma

Iowa State University

Ames, Iowa

2004

Graduate College
Iowa State University

This is to certify that the master's thesis of

David A. Olds

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy

DEDICATION

I must express my deepest gratitude to my parents, Drs. John and Rosemary Olds. My parents always encouraged me to work hard to become the best at whatever I undertook. Their unconditional love and support inspired me to accomplish my goals and helped my dreams to come to life. I also dedicate this study to the 12 million people in the United States of America who have chosen the restaurant industry (the nation's largest employer outside the government) as their life's work.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Food safety remains a concern in commercial and institutional foodservice operations. Proper cooling of cooked food products has been shown to significantly reduce the chance of outbreaks of foodborne illness, yet cooling often is done incorrectly. Further, equipment for optimal cooling frequently is not available in foodservice operations, and time and temperatures during the cooling process are seldom checked. The purpose of this study was to determine the impact of various cooling methods used in foodservice on the microbiological safety of meat products. Specific objectives include comparing four different cooling methods for cooked USDA turkey roasts artificially inoculated with *Clostridium perfringens* spores and six different cooling methods for chili. In addition, this study documented time and temperature continuously through the cooling process to determine if food cooling standards are being met.

Typical cooling methods failed to meet food cooling time standards. For example, turkeys cooled in whole roast form under typical foodservice refrigeration showed an unacceptable and dangerous increase of up to 4.00 $\log_{10}$ CFU/roast in viable *C. perfringens* counts. No growth of *C. perfringens* occurred in roasts divided into quarters and cooled under typical foodservice refrigeration and blast-chilled whole turkey roasts. An acceptable and beneficial reduction (-2.7 $\log_{10}$ CFU/roast) in viable *C. perfringens* counts was observed when quartered and blast-chilled methods were used.

None of the cooling methods for turkey achieved established time and temperature guidelines but yet the quartered roast treatment and the blast chilled

roast treatment both showed reduction in viability of *C. perfringens*. This may indicate that the established cooling guidelines are too stringent for foodservice operations.

Chili, blast chilled in two-inch and four-inch deep counter pans, met cooling guidelines, cooling from 135°F to 70°F (57°C to 21°C) and from 70°F to 41°F (21°C to 5°C) in approximately two and four hours, respectively. However, chili cooled using the same sized pans under typical foodservice refrigeration did not. Chili cooled in 3-gallon increments under typical foodservice refrigeration (39°F [3.9°C]) showed enormous violations of food cooling guidelines, taking approximately 24 hours to cool from 135°F to 41°F (57°C to 5°C). With the use of a chill stick, the same 3-gallon container of chili cooled under typical foodservice refrigeration showed a 75% reduction in cooling time to approximately six hours. Further research is needed using typical cooling methods with different food products and foodservice operators need to implement appropriate cooling methods to ensure proper cooling of potentially hazardous foods.

CHAPTER 1. INTRODUCTION

Food safety has become an important issue in the United States. More than 28 million children are served lunch each day through the National School Lunch Program and 8 million children participate each day in the School Breakfast Program (USDA, 2003). Furthermore, four of ten adults patronize restaurants daily (NRA, 2002a). Foodservice owners and operators increasingly are faced with maintaining high standards of food quality and food safety within their operations.

The Centers for Disease Control and Prevention (CDC) estimated that foodborne illnesses (FBI) cause "approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year. Known pathogens account for an estimated 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths....while unknown agents account for the remaining 62 million illnesses, 265,000 hospitalizations, and 3,200 deaths" (Mead et al., 1999). McNab (1998) estimated the cost of microbial food poisoning in the United States at $5 billion to $6 billion annually.

Between 1973 and 1999, a reported total of 15,831 foodborne illness outbreaks resulted in 447,483 cases of FBI, 20,119 hospitalizations, and 457 fatalities (U.S. GAO, 2003). Of these 15,831 reported outbreaks, 53.5% originated in restaurants; 15.2% in private homes; 3.5% in schools; 23.4% in other locations; and 4.5% were of unknown origin (U.S. GAO, 2003). The USDA has estimated FBI to cost the U.S. between $7 billion and $37 billion annually (U.S. GAO, 2003).

Despite a projected increase in restaurant sales from $426.1 billion in 2002, to $1 trillion in 2010, customer confidence in food safety is on the decline. Allen

(2000) stated that 50% of consumers rated the restaurant industry's ability to ensure food safety as excellent in 1995 compared to 39% in 2000. Customer perceptions of food safety and FBI are influenced by media accounts of food-related outbreaks and the resulting publicized aftermath of expensive litigation and ruinous financial consequences.

In retail foodservice operations, many food handling practices may result in unsafe food. Correct cooking and food handling methods help control outbreaks of foodborne illness by preventing contamination of food products that can cause FBI. Bryan (1988) found that improper cooling of food was the leading factor contributing to outbreaks of FBI, accounting for 44% of 1,918 outbreaks in the U.S. from 1961 to 1982. Proper cooling and food handling methods employed after the cooking of food products help to control both temperature-related microbiological growth in food and the resulting diseases transmitted to humans through the consumption of these pathogen-laden foods.

The *Report of the FDA Retail Food Program Database* (FDA, 2000) stated that 85% of full-service restaurants were not in compliance with FDA standards for cooling potentially hazardous foods (PHF) from 140°F to 70°F (60°C to 21°C) in two hours and then from 70°F to 41°F (21°C to 5°C) in an additional four hours. In a study to evaluate readiness for implementation of Hazard Analysis Critical Control Point (HACCP) systems, Henroid and Sneed (2004) observed that in ten of forty school foodservice operations in which cooling was observed, six were not in compliance with FDA standards of cooling PHF. Documentation of temperatures by

three-fourths of the observed foodservice operations was poor, casting doubt upon their ability to cool PHF within the Food Code time standards.

Food not cooled within this time period is susceptible to rapid growth of pathogenic bacteria that survive the cooling process, increasing the potential for FBI. The cooling of meat and poultry products within these FDA guidelines is especially crucial due to the presence of spore-forming heat-resistant bacteria (Steele & Wright, 2001).

Spores of foodborne bacteria that are associated with meat, such as *Clostridium perfringens,* are very heat-resistant and usually cannot be killed during cooking without destroying the quality of the food product (Doyle, 2002). *C. perfringens* spores that survive cooking may be activated and begin producing vegetative cells if cooling is slow enough (Doyle, 2002). Therefore, the quick cooling of cooked meat products is an important standard operating procedure for all retail foodservice operations in order to prevent FBI.

Purpose in the Study

The purpose in this study was to determine time and temperature curves and microbiological safety of meat products cooled using various cooling methods observed in foodservice operations. Specific objectives included:

1. Develop time and temperature cooling curves for USDA commodity turkey roasts using four cooling methods: quartered roasts cooled in walk-in refrigerator; whole roast cooled in a blast chiller; whole roast loosely wrapped and cooled in walk-in refrigerator; and three whole roasts placed closely together on a sheet pan, tightly bagged, and cooled in a walk-in refrigerator.

2. Compare the growth of *C. perfringens* in artificially inoculated turkey roasts that were heated to an internal temperature of 74°C and cooled using four cooling methods.
3. Develop time and temperature cooling curves for chili cooled using six cooling methods: 2" and 4" depth in counter pans in walk-in refrigerator; 2" and 4" depth in counter pans in blast chiller; stockpot in walk-in refrigerator; and stockpot with chill stick in walk-in refrigerator.

Significance of the Study

Improper cooling of food products is the number one factor contributing to outbreaks of FBI in the retail foodservice industry. This study directly addresses this issue and provides some insight into cooling-related FBI outbreaks. This research study provides data to: (a) develop educational recommendations for improving the practices of food handlers; (b) update and verify effective cooling methods for foodservice operations; and (c) continue research on foodservice cooling methods.

Limitations of the Study

Only two meat products (turkey roasts and ground beef) were studied. For safety and validity reasons, a microbiological laboratory was used to conduct the microbiological analysis. Although the simulation of actual foodservice cooling procedures occurred, the exact duplication of a retail foodservice operation within the confines of a laboratory was not feasible. Furthermore, variables related to food handlers, such as handwashing frequency and timing, were stringently controlled in the laboratory setting and may not have replicated actual practices.

Definition of Terms

Blast Chiller: A specialized cooling device that uses forced, chilled air to cool food rapidly. Rapid cooling helps prevent foodborne illness by preventing microbial growth in the temperature danger zone (TDZ).

Clostridium perfringens: A species of the genus *Clostridium,* which contains a group of bacteria that do not thrive in an oxygen-rich environment and have the ability to create heat-resistant endospores. These anaerobic bacteria can be pathogenic for both animals and humans. *C. perfringens* is found widely in the environment, especially in soil and areas of fecal contamination, and also survives in the intestines of both humans and animals. *C. perfringens* is an anaerobic, gram positive, spore-forming rod that is the most abundant toxin-producing species in the clostridial group (Heredia & Labbé, 2001).

Clostridium perfringens enterotoxin (CPE): A toxin that is released by *C. perfringens* vegetative cells undergoing sporulation (formation of spores), usually in the small intestine. CPE can cause diarrhea, cramping, and severe abdominal pain.

Foodborne Illness (FBI): Foodborne illnesses are defined as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food.

Germination: The process by which bacterial spores shed their protective spore covering, and become vegetative cells.

Heat Shocked: When a *Clostridium perfringens* spore is exposed to high levels of heat (75°C) for a period of time (20 minutes), it is triggered (heat-shocked) to germinate. When the triggered (heat-shocked) spore cools to a favorable

temperature (between 37°C and 45°C), it germinates, sheds the spore, and becomes a vegetative cell.

Potentially Hazardous Food (PHF): "a food that is natural or synthetic and requires temperature control because it is in a form capable of supporting:

- The rapid and progressive growth of infectious or toxigenic microorganism;
- The growth of toxin production of *Clostridium botulinum;* or
- In raw eggs, the growth of *Salmonella enteritidis*" (FDA Food Code 2001, p. 420).

Retail Foodservice: Three distinct industry segments comprise retail foodservice: (a) Institutional foodservice establishments (hospitals, nursing homes, elementary schools); (b) Restaurants (fast food, full-service); & (c) Retail Food Stores (Deli departments, meat and poultry departments, produce departments and salad bars, seafood departments) (FDA, 2000).

Spore: A protective covering surrounding bacteria. Spore-forming bacteria can create spores (sporulate) to protect themselves or produce vegetative cells from spores (germinate) when conditions are favorable for growth. The ability of *Clostridium perfringens* to form heat-resistant spores means that it can survive most cooking processes.

Sporulation: The process by which vegetative bacteria form spores to protect themselves. It is during *Clostridium perfringens* sporulation that enterotoxins are released, which can cause foodborne illness (FBI).

Temperature Danger Zone (TDZ): The Temperature Danger Zone (TDZ) refers to the range of temperatures between 41°F (5°C) and 135°F (57°C). Potentially

hazardous food (PHF) is susceptible to rapid microbial growth within the TDZ; careful heating and cooling procedures must be followed to keep PHF out of the TDZ. It should be noted that the TDZ was 41°F (5°C) to 140°F (60°C) prior to the amended 2001 Food Code; thus, depending on the time of the study reported, either standard may be reported.

Vegetative Cell: With regards to *C. perfringens*, a vegetative cell is one that has shed its protective spore covering. Vegetative cells, free of their protective coating, can multiply rapidly. However, unlike spores, vegetative cells cannot withstand high levels of heat.

CHAPTER 2. REVIEW OF LITERATURE

Adequate cooling of potentially hazardous foods is important in retail foodservice to ensure the safety of food served to consumers. This review of literature will address the following areas as they relate to cooling: foodborne illness, observational cooling studies, laboratory cooling studies, and preventative food safety measures in retail foodservice.

Foodborne Illness

Pathogens

Over 200 diseases are transmitted through food via agents such as bacteria, viruses, parasites, metals, toxins, and prions (Mead et al., 1999). The *Norwalk-like virus* accounts for 66.7% of FBI in the United States. *Campylobacter jejuni,* a gram-negative microaerophilc bacterium, causes 14.2% of FBI in the U.S. Other pathogens causing FBI (in descending order by percentage of FBI) include: *Salmonella* (9.7%); *Clostridium perfringens* (1.8%); *Giardia lamblia* (1.4%); *Staphylococcus aureus* (1.3%); *Escherichia coli* O157:H7 (1.3%); *Shigella* (0.6%); *Streptococcus* (0.4%); *Hepatitis A virus* (<0.1%); and *Listeria monocytogenes* (<0.1%) (FDA/CFSAN, 2003). These pathogens, consumed with contaminated food, can transmit FBI to an individual with varying degrees of severity.

Common Symptoms of FBI

Anyone can get sick from consuming contaminated food; however, there are many variables that can affect an individual's susceptibility to contracting FBI. Age, health, quantity of consumed contaminated food, and virulence of the pathogenic organism all are variables that affect the likelihood of getting FBI. Common

symptoms of FBI include vomiting, abdominal discomfort, diarrhea, and nausea. Susceptible at-risk individuals may become very sick and can even die from FBI because their immune systems are not capable of defending against the bacteria (FDA/CFSAN, 2003). An individual's defense against FBI also is related to: (a) the type of food involved; (b) its associated pathogen; and (c) whatever improper food-handling procedures may have occurred before the individual consumed the pathogen-laden food.

Types of Foods Associated with FBI

Almost any food can become contaminated if stored, prepared, or handled incorrectly. Foods rich in protein, including meat, fish, and poultry, commonly are involved in FBI outbreaks for two reasons: (a) protein-rich foods are usually of animal origin and microorganisms of animal origin often are found in animal foods; and (b) protein-rich animal foods are broken down into amino acids by bacteria and these amino acids are an important nutrient source for bacteria (FDA/CFSAN, 2003). As time elapses and bacteria multiply, so does the chance for contracting FBI as a result of consuming these relatively high numbers of pathogens in protein-rich foods. Each type of food is associated with certain types of FBI-causing pathogens. However, the rate of pathogenic growth is not caused solely by the type of food involved, but also by the choice of food-handling practices.

Food Handling Practices and FBI in Retail Foodservice

In retail foodservice operations, food handling practices may result in unsafe food, a major concern for both customers and foodservice operators. Retail food handling practices are significant, especially in light of the $426.1 billion of retail

foodservice revenues generated in the U.S. annually (NRA, 2002b). Forty cents of every food dollar is directly spent on the 54 billion meals eaten in restaurants and school and work cafeterias in the U.S. each year (NRA, 2002a). These meals are produced and served (handled) by the 11.7 million employees in the foodservice industry, the largest private-sector employer in the U.S. (NRA, 2002b). Food handling practices are directly related to the majority of outbreaks of FBI in the retail foodservice industry.

Bryan (1988) found the top three factors that contributed to the occurrence of 1,918 outbreaks of foodborne illness (by rank) between 1961 and 1982 were: (a) improper cooling of food (44%); (b) a lapse of twelve or more hours between preparing and eating (23%); and (c) a colonized person handling implicated food (18%). The top two factors, accounting for 67% of all outbreaks, are time and temperature related - the two fundamental elements of the cooling process.

Proper cooling and food handling methods employed after the cooking of food products help to control both the temperature-related microbiological growth in food, and the resulting illnesss transmitted to humans through the consumption of these pathogen-laden foods. Observational and laboratory studies have shown the importance of proper cooling procedures for foods.

Observational Cooling Studies

Observational studies allow researchers to study "things as they are," which is very valuable in foodservice research. Bryan (1988) identified inadequate cooling as being associated with most of the FBI outbreaks from 1961-1982. In addition, Bryan also stated that a lapse of twelve or more hours between preparation and

consumption of food and colonized individual's handling of food also contribute (albeit a lesser degree than improper cooling) to FBI outbreaks. Examples of improper cooling methods included holding foods at room temperature for long periods of time and storing foods in large/deep containers in refrigerators.

"The Food and Drug Administration (FDA) is responsible for setting standards for the safe production of foods and advising state and local governments on food safety standards for institutional food service establishments, restaurants, retail food stores and other retail food establishments" (FDA, 2000). In response to a 1996 report identifying foodborne illness to be a "significant public health problem" (FDA, 2000) in the U.S., the FDA established a National Retail Food Steering Committee, which included representation from a number of governmental organizations.

The purpose of this steering committee was to measure the occurrence of foodborne illness risk factors, including: (a) food from unsafe sources; (b) inadequate cooking; (c) improper holding temperatures; (d) contaminated equipment; and (e) poor personal hygiene. Data were gathered from 895 inspections of restaurants, institutional foodservice establishments, and retail stores, yielding 17,477 observations.

The *Report of the FDA Retail Food Program Database* (FDA, 2000) reported that 85% of full-service restaurants were not in compliance with FDA standards of cooling potentially hazardous foods (PHF) from 140°F (60°C) to 70°F (21°C) in two hours and then from 70°F (21°C) to 41°F (5°C) in an additional four hours. The

sample size from other sectors, such as schools and health care, was too small to draw conclusions on cooling practices.

Henroid and Sneed (2004) observed food handling practices of employees in a convenience sample of 40 schools from 40 school districts. Most of these foodservice operations were not taking food temperatures, and in those operations in which food temperatures were taken, temperatures were not documented. Many employees indicated that they did not calibrate their thermometers, and calibration documentation was non-existent in all of the schools studied. Henroid and Sneed also observed that six of 10 school foodservice operations were not in compliance with FDA standards of cooling potentially hazardous foods (PHF) from 135°F (57°C) to 70°F (21°C) in two hours and then from 70°F (21°C) to 41°F (5°C) in an additional four hours. Documentation of temperatures by three-fourths of the observed foodservice operations was poor, casting doubt upon their ability to cool potentially hazardous foods (PHF) from 135°F (57°C) to 70°F (21°C) in two hours and then from 70°F (21°C) to 41°F (5°C) in four hours.

In a GAO Report to Congressional Requestors, state health officials in 32 states were surveyed using a web-based instrument regarding 97 outbreaks involving 50 or more individuals between 1990 and 1999 (U.S. GAO, 2002). Results indicated that meat dishes were the number one link to FBI outbreaks, and that *C. perfringens* showed strong epidemiological support as a suspected causative FBI outbreak agent. Improper cooling and food handling methods also were identified as an underlying cause of some FBI outbreaks.

Overview of *Clostridium perfringens*

Clostridium perfringens is a species of the genus *Clostridium,* which contains a group of bacteria that do not thrive in an oxygen-rich environment and have the ability to create heat-resistant endospores. These anaerobic bacteria can be pathogenic for both animals and humans. *C. perfringens* is found widely in the environment, especially in soil and areas of fecal contamination, and also survives in the intestines of both humans and animals. *C. perfringens* is an anaerobic, gram positive, spore-forming rod that is the most abundant toxin-producing species in the clostridial group (Heredia & Labbé, 2001).

The toxins produced by *C. perfringens* during spore formation may cause a large variety of human and animal diseases, some of which can be potentially (but rarely) deadly. Most outbreaks of *C. perfringens* occur when food is cooked and improperly cooled. *C. perfringens* is especially of concern to retail foodservice, where large amounts of food are cooked, cooled, and held until service. Violations of the FDA 2001 Food Code guidelines for recommended proper cooling times can result in a foodborne outbreak, imposing the burden of accountability upon the retail foodservice operator.

Characteristics of Clostridium perfringens

C. perfringens has many attributes that enable it to cause serious foodborne illness. First, *C. perfringens* is found throughout the natural environment, in the soil, the air, and in animal feces and hides; thus, *C. perfringens* is omnipresent in its ability to contaminate humans, animals, and food. Second, *C. perfringens* is able to form heat-resistant spores that allow it to survive high levels of heat (i.e. during

cooking or incomplete sterilization) that would kill most organisms. Third, *C. perfringens* multiply rapidly in the Temperature Danger Zone (TDZ: 41°F - 135°F [5°C - 57°C]), which can produce high microbial levels encountered in food poisoning. Fourth, *C. perfringens* can produce an active enterotoxin that is responsible for abdominal cramps, intestinal gas, and diarrhea (Heredia & Labbé, 2001).

Clostridium perfringens and Retail Food Safety Issues

C. perfringens food poisoning is an ongoing concern in retail foodservice, where large volumes of food product are prepared in advance and cooled before reheating for service. Spores that exist naturally in processed meats (e.g. turkey roasts and ground beef) survive and are heat-activated during the cooking process, while other vegetative bacteria are killed off at temperatures of 165°F (74°C) or above (Heredia & Labbé, 2001). The problem with *C. perfringens* occurs when these thick, large volume food products are not cooled down quickly enough and not brought through the TDZ (41°F - 135°F [5°C - 57°C]) rapidly.

Unfortunately, *C. perfringens* spores take advantage of these prolonged periods of warmth and (after heat activation during cooking) they begin producing vegetative *C. perfringens* cells that multiply rapidly. Human ingestion of a high level ($>10^5$ CFU/g) of vegetative *C. perfringens* cells can overwhelm the balance of flora in the small intestine. When the *C. perfringens* levels are high enough to survive the acidity in the stomach, the vegetative cells enter the small intestine. After entering the small intestine, vegetative *C. perfringens* cells begin to sporulate, producing

Clostridium perfringens enterotoxin (CPE), which will be released into the small intestine along with mature *C. perfringens* spores (Heredia & Labbé, 2001).

Once CPE is released into the small intestine, it causes localized tissue damage, and disrupts fluid and electrolyte transport properties in the intestine, leading to diarrhea and severe abdominal pain (Heredia & Labbé, 2001). Health inspectors gathering data from a suspected *C. perfringens* outbreak would use at least one of three methods for detection: (a) $>10^5$ vegetative cells/g in the suspected food; (b) $>10^6$ spores/g in the feces of the infected individuals; or (c) direct identification of CPE in stool samples of the infected individuals (Heredia & Labbé, 2001).

Laboratory Cooling Studies

Several experimental studies have been conducted using time, temperature, and microbiological analyses to explain the effect of cooling upon cooked food. Juneja, Snyder, and Cygnarowitz-Provost (1994) studied the ability of *C. perfringens* spores to germinate and grow to determine a safe cooling rate for cooked beef. Ground beef samples were inoculated with heat-shocked *C. perfringens spores,* vacuum-packaged, and cooked in a water bath to 60°C (140°F) in one hour. The samples were then cooled and samples were removed at various points during the cooling process, from 54.4°C (130°F) to 7.2°C (45°F); microbiological analysis was performed on each of the cooled samples.

These researchers found that minimal microbiological growth occurred in *C. perfringens* samples with cooling periods of up to fifteen hours. Samples that took longer than eighteen hours to reach 7.2°C (45°F) showed an increase from an

inoculum of 1.5 $\log_{10}$ to 6.0 $\log_{10}$, a potentially hazardous infective dose level of *C. perfringens.*

Bacterial spore formation occurs only under adverse conditions, such as drying or nutrient depletion. Spores have very thick walls and are resistant to (a) heat, (b) chemicals, (c) food irradiation, (d) drying, and (e) cooking. Spore destruction occurs during autoclaving, under 15 psi at 121.1°C (250°F) for 15-20 minutes, an environment that would never occur during normal cooking of turkey roasts at 74°C (165°F) (Ray, 2001).

Steele and Wright (2001) identified the growth potential of *C. perfringens* spores in ready-to-eat cooked turkey products. Raw turkey breasts were inoculated with *C. perfringens* spores and cooked in a steam oven to an internal temperature of 72°C (161.6°F). Various samples were cooled to 7.2°C (45°F) within six, eight, and ten hour intervals and microbiological analyses were performed on each of the cooled samples.

Steele and Wright (2001) compared their findings to the USDA Food Safety Inspection Service (USDA/FSIS) safe cooling standard criteria (1999), allowing no more than a 1 $\log_{10}$ increase between 48.9°C (120°F) and 12.8°C (55°F) within six hours as a measure of prevention of the occurrence of potential *C. perfringens* outbreaks. They found that in actuality, a cooling period of no greater than 8.9 hours should be used to prevent potential *C. perfringens* outbreaks, 2.9 hours longer than recommended by the USDA/FSIS.

Snyder (1997) challenged the ability of retail foodservice operations to comply with the 1976 FDA food code, which called for food to be cooled from a hot

state to 7.2°C (45°F) in four hours. Snyder also makes the same challenge in regards to the 1997 Food Code, which calls for food to be cooled from 60°C (140°F) to 21.1°C (70°F) in two hours and from 21.1°C (70°F) to 5°C (41°F) in four hours. Snyder showed that no commercial NSF International refrigerator would cool food two inches of depth in four hours, according to either the 1976 or the 1997 FDA Food Codes.

Snyder and Labalestra (2004) further explained the difficulties involved in meeting 1999 FDA Food Code requirements. They argued that foodservice and restaurant operators lack: (a) the ability to accurately measure the cooling process, which is "very complicated"; (b) the ability to gather accurate data and determine if the cooling has been done in six hours; and (c) an understanding of the mathematics of cooling.

Preventative Measures in Retail Foodservice

When *C. perfringens* foodborne outbreaks occur, any or all of the following events may have transpired: (a) improper cooling of food; (b) inadequate reheating of food; (c) preparation of food more than 24 hours in advance of service; and (d) improper hot holding of food. Food safety remains a concern to commercial and institutional foodservice operators. Improper cooling of cooked food products has been identified as the number one cause of FBI (Bryan, 1988). Proper cooling of cooked food products has been shown to significantly reduce the chance of outbreaks of foodborne illness; yet cooling often is done incorrectly. Further, equipment for optimal cooling frequently is not available in foodservice operations,

and time and temperatures during the cooling process are seldom checked (Henroid & Sneed, 2004).

Between 1973 and 1999, a reported total of 15,831 foodborne outbreaks effectively caused 447,483 cases of FBI, 20,119 hospitalizations, and 457 fatalities (U.S. GAO, 2003). *C. perfringens* has been implicated in 11.8% of the total bacterial foodborne illness outbreaks in the United States and 20.6% in Canada (Juneja, Snyder & Cygnarowicz-Provost, 1994). To control these outbreaks, preventative measures need to be taken by retail foodservice operators to ensure the well-being of society.

Improper Cooling

The *Report of the FDA Retail Food Program Database* (FDA, 2000) provided estimates that 85% of full-service restaurants were not in compliance with FDA standards for cooling potentially hazardous foods (PHF) from 140°F (60°C) to 70°F (21°C) in two hours and then from 70°F (21°C) to 41°F (5°C) in an additional four hours. *C. perfringens* thrives well during prolonged cooling, which makes it essential that retail foodservice operators do all that is necessary to meet FDA guidelines.

Because large quantities of food cool slowly, it is imperative to employ effective methods to lessen food's exposure to the TDZ. This can be done in a number of ways:

1. Separate or cut food products into smaller portions before refrigeration.
2. Rapidly cool food products using specialized equipment such as:
    a. Ice bath
    b. Blast Chiller

3. Pour liquid foods, such as chili, into shallow pans to increase evaporative area, thus bringing the temperature down more quickly under refrigeration.

Inadequate Reheating

The FDA 2001 Food Code states that potentially hazardous food that is cooked, cooled, and reheated for hot holding shall be reheated so that all parts of the food reach a temperature of at least 165°F (74°C) for 15 seconds. This will ensure that the vegetative *C. perfringens* cells are destroyed, although *C. perfringens* spores will remain. This is especially important in the case of gravy, which is often (improperly) reused day after day, and is "conveniently" put in the steam table for "reheating."

Improper Hot Holding

The FDA 2001 Food Code also states that food (once cooked or reheated), must be held at 140°F (60°C) or above. *C. perfringens* often is implicated in outbreaks at institutional foodservice establishments, where large quantities of food used to feed large numbers of people sometimes remain at temperature levels below 140°F (60°C), an ideal zone for *C. perfringens* growth.

Preparation of Food More Than 24 Hours in Advance

In large retail foodservice establishments, especially high volume institutional operations that utilize banquet-style service, food is often prepared well in advance. *C. perfringens* may grow if food prepared moer than 24 hours in advance does not completely cool, and is reheated incorrectly and/or held at a low temperature (Heredia & Labbé, 2001).

Example of Failure to Implement Proper Preventative Food Safety Measures

According to Ray (2001), in 1989, 300 of 420 corporate employees became ill following a luncheon the day before. Abdominal cramps, diarrhea, and nausea were symptoms reported from 113 people interviewed. It was determined that 98% of the ill employees had dined on roast beef, while those who had eaten chicken had not become ill.

The beef roasts, which weighed 19 pounds, were cooked three days before the banquet, and stored in the refrigerator. The likely chain of food preparation is as follows:

1. Roasts were prepared too far in advance, cooked, cooled (in whole form), and were allowed to stay in the TDZ too long, allowing the germination of *C. perfringens* spores and subsequent growth of *C. perfringens* vegetative cells to dangerous levels ($>10^5$ CFU/g).
2. The roasts were likely reheated in their whole form, rather than slicing and shingling into shallow hotel pans. This likely resulted in the internal temperature of the roasts not reaching a temperature of at least 74°C (165°F) for 15 seconds.
3. The roasts were served or held at a temperature level below 60°C (140°F).
4. The employees consumed the roasts, containing a level of $>10^5$ CFU/g of vegetative *C. perfringens,* which overwhelmed the balance of flora in the employees' intestines. The cells then sporulated, released CPE, and the employees became ill.

Retail food safety depends upon conscientious management by both managers and hourly employees. By implementing food safety training programs, such as ServSafe® *Essentials* (NRAEF, 2004), which explain "why" it is important to obey health code rules and regulations, an understanding of *C. perfringens* behavior in susceptible food products may be reached and may help to prevent future foodborne outbreaks. Preventative measures include: (a) use of proper cooling techniques; (b) monitoring cooking and holding temperatures; and (c) not preparing food more than 24 hours in advance. Following these guidelines should help reduce the instances of retail foodservice *C. perfringens* foodborne outbreaks.

Summary

In a review of the scientific literature and inspection reports, Snyder and Labalestra (2004) found a lack of evidence that health departments were actually performing cooling studies to verify six hour cooling in retail foodservice. Snyder further stated that a six-hour cooling time is a very difficult standard to meet under typical foodservice refrigeration. The gap between stringent FDA cooling recommendations and verification of cooling standards, coupled with observations of non-compliance in retail foodservice cooling procedures, served as impetus for this study. Further, there is a paucity of research on time, temperature, and microbial growth of *C. perfringens* using commonly observed retail foodservice cooling methods with raw turkey roasts. Accordingly, the objectives of this research were two-fold. First, using a commonly-prepared food product in retail foodservice, this study evaluated the influence of selected retail foodservice methods on the

behavior of *C. perfringens* ATCC 10388 in turkey roasts following heating to an internal temperature of 74°C.

Second, in addition to turkey, chili (another commonly-prepared food in retail foodservice) was evaluated using time and temperature cooling curves for six treatments of chili cooled using three different types of cooling equipment: a walk-in refrigerator; a blast chiller; and a chill stick. By evaluating cooling methods for two food products common to retail foodservice, this study provided insights into addressing factors involved in cooling-related FBI outbreaks (such as growth of *Clostridium perfringens*) and produced results that could: (a) develop educational recommendations for improving the practices of food handlers; (b) help update and/or verify effective cooling methods for foodservice operations; and (c) provide data for continuing research on foodservice cooling methods.

CHAPTER 3. MATERIALS AND METHODS

Turkey Roasts

Test Organism

Clostridium perfringens spore strains ATCC 10388, NCTC 8238, and NCTC 8239 were secured from the culture collection of Iowa State University. The organisms were maintained at 39.2°F (4°C) in cooked-meat medium (Difco Laboratories, Detroit, MI). *C. perfringens* strains NCTC 8238 and NCTC 8239 were pilot tested and were eliminated from the study after they were determined to be inconsistent in their culturability under the conditions used in this study. Only *C. perfringens* strain ATCC 10388 was used in the study as the target organism.

Preparation of Inoculum

Inoculum was prepared using procedures described by Juneja, Call, and Miller (1993). These procedures are described in detail in Appendix A, Procedures 1, 2, and 3. To prepare inocula, 0.1 ml of *Clostridium perfringens* strain ATCC 10388 from the stock culture was transferred into 10 ml freshly prepared fluid thioglycollate medium (FTM). The inoculated FTM was heat shocked at 167°F (75°C) for 20 minutes and incubated at 98.6°F (37°C) for 18 hours.

After 18 hours, 1.0 ml of the incubated FTM inoculum was transferred into 10ml of freshly steamed FTM and incubated at 98.6°F (37°C) for four hours to produce growth of vegetative cells. After four hours, 1.0 ml of the incubated FTM inoculum was transferred into Duncan Strong (DS) sporulation media (Duncan & Strong, 1968) and incubated at 98.6°F (37°C) for 24 hours. Duncan Strong inoculum was diluted in physiological saline (0.85%). The inoculum was dyed with four drops

of a green food grade dye per 10 ml of inoculum immediately before injection into turkey roasts.

Sample Preparation and Inoculation

Forty 11.25 pound USDA commodity boneless symmetrical tube-shaped Jennie-O frozen turkey roasts (more than 93% water with not more than 5.5% dissolved phosphates and aids in mixing, not more than 1.5% salt, not more than 0.5% sodium phosphates) were secured from the USDA's Food, Nutrition, and Consumer Services and kept in cold storage at 0°F (-17.7°C) until required for use. Prior to cooking, roasts were placed in a walk-in refrigerator at 39.2°F (4°C) for two days until roasts were completely thawed.

All roasts were inoculated using the procedure as described in Appendix A, Procedure 5 with a slight modification for treatment 1. Thawed turkey roasts measuring 14 cm x 40 cm were inoculated by injecting 1 ml of the dyed spore inoculum with a 6-inch (15.24 cm) 20-gauge needle in the center of the roast. The inoculum depth of approximately 7 cm per roast was determined by measuring the circumference of the respective roasts and applying the mathematical formula $C = \pi D$ to obtain the radius (½D), which indicated the depth of the center of the roast in which to inoculate.

Thermal type-K probes were inserted next to the inoculum at the same depth as the inoculum (approximately 7 cm), in the center of the turkey roast. Each roast was vacuum packed using a Multivac model A 300/51 vacuum packaging machine. Rival VSB2 Seal-a-Meal® plastic bag material was used, allowing the type-K probe to remain securely in place, and to facilitate cooking without allowing air or liquid to

enter. Type-K probes were attached to an Atkins model #37313 data recording digital thermometer set to log temperature data at 10 minute intervals.

Pilot Testing

Cooking, cooling, and enumeration procedures were pilot tested. During pilot testing, all treatments showed measurable increases or reductions of *C. perfringens*. Treatment 1 showed particularly large reductions in counts, sometimes falling below the lowest detectable dilution level (10^{-2}). To observe this more closely, two extra roasts were used to supplement treatment 1 for replications two and three.

Cooking Procedures

Three replications of each treatment were performed. The same procedures were used for each replication. All roasts were prepared and inoculated according to the previously stated sample preparation and inoculation guidelines and cooked in a steamer at 212°F (100°C). All roasts were cooked to the same end-point temperature of 165°F (73.9°C), according to the 2001 FDA Food Code standard.

Cooling Procedures

Upon removal from the steamer, roasts were placed under one of four cooling treatments. In all treatments, a type-K probe (one for each cooling method) was attached to an independent data recording thermometer to record cooling temperatures over time, until cooling was completed at 41°F (5°C).

Treatment Summary

The cooling treatments for the turkey roasts are summarized in Table 1. For treatment 1, one roast was inoculated using a slightly modified procedure. The 40

cm long roast was marked into four 10 cm quadrants. The roasts were inoculated with 1 ml of the dyed *C. perfringens* cocktail mixture at a depth of approximately 7 cm in the center of one of the inner two quadrants. A type-K probe was inserted into the center of the other inner quadrant at a depth of approximately 7 cm. The roast was cooked whole and cut into four 10 cm thick pieces (quadrants) prior to cooling. Each section was spread apart, leaving a gap of approximately 7 cm from the other three sections for cooling, uncovered, in a walk-in cooler.

Table 1. Turkey Cooling Treatments

	Number of turkey roasts Per replication	Total
1. One roast quartered, cooled in walk-in cooler	1	3
2. One roast loosely wrapped, cooled in blast chiller	1	3
3. One roast loosely wrapped, cooled in walk-in cooler	1	3
4. Three roasts bagged, cooled in walk-in cooler	3	9
Pilot Test Roasts used:		11
Roasts used to supplement treatment 1:		4
Total Roasts used:		33

For treatment 2, one roast was inoculated, fitted with a type-K probe, cooked, and cooled, loosely covered, in a blast chiller. For treatment 3, one roast was inoculated, fitted with a type-K probe, cooked, and cooled, loosely covered, in a walk-in cooler. For treatment 4, three cooked roasts were placed closely to one another on a sheet pan and the entire sheet pan was tightly covered with a plastic bag during cooling. The middle roast was considered the roast most likely to take

the longest time to cool and was the roast that was inoculated and fitted with a type-K probe.

Microbiological Analysis

After the internal temperature of the turkey roasts reached 41°F (5°C) all the turkey roasts were cut open using an aseptic knife and prepared for microbiological analysis as described in detail in Appendix A, Procedure 6. In an attempt to recover all viable vegetative *C. perfringens* cells from the core of each roast, all green-dyed cooked meat was aseptically excised from the surrounding meat. The dyed meat was placed in Fisher Brand Filtra Bags, macerated by hand, and mixed with 100 ml sterile 0.1% peptone water. The dyed meat and peptone mixture was pummeled for 60 seconds with a Seward 400 Lab System Stomacher at normal speed.

From the resulting meat slurry, dilutions ranging from 10^{-2} to 10^{-8} were prepared. All dilutions were transferred into individual Fisher Petri dishes and pour plated with Shahidi-Ferguson-Perfringens (SFP) agar base enriched with a filter-sterilized D-Cycloserine solution. SFP D-Cycloserine inoculated plates were incubated anaerobically at 37° for 24 hours and bacterial colonies on agar plates were counted after incubation via use of a Quebec counter. The resulting CFU/roast counts of *C. perfringens* were derived from colony counts of typical *C. perfringens* colonies.

Data Analysis

Three independent replicate experiments were conducted and microbiological counts were recorded for all replications. Time and temperature curves were developed from data downloaded from Atkins #37313 data recording

thermometers using Microsoft Excel 2003. Means and standard deviations of all microbiological counts and time and temperature data were calculated.

Chili

Sample and Sample Preparation

Chili con carne with beans was prepared using a recipe from *USDA Recipes for Child Nutrition Programs* obtained from the National Food Service Management Institute website (NFSMI, 1999). USDA commodity ground beef was secured for the study.

Cooking Procedures

Chili was prepared in a quantity food production laboratory at Iowa State University, using a 15-gallon (56.78 liter) Cleveland tilting skillet. All recipe ingredients and cooking procedures were followed exactly, under carefully controlled laboratory conditions.

Cooling Procedures

After cooking, the chili was transferred while still hot (190°F [87°C]) into three different sized containers for cooling. Three quarts (2.84 liters) of hot chili were placed into a 12"x10"x 2 ½" stainless steel pan. Five quarts (4.73 liters) of hot chili were placed into a 12"x10"x4" stainless steel pan. Three gallons of hot chili (11.36 liters) were placed into a stainless steel stockpot. The same stainless steel containers were used for all three replications of the experiment.

A type-K probe was placed in the geometric center of the chili to measure the hottest area of the container during cooling. The type-K probe was attached to an

Atkins model #37313 data recording thermometer programmed to log temperatures at 10-minute intervals.

Chili was cooled uncovered, with the doors to the cooling equipment closed during the entire cooling process. These practices represented a "best case" scenario for the cooling of chili, and did not take into account variables in actual foodservice establishments that could potentially increase cooling time, such as opening and closing a refrigerator door or wrapping hot food tightly with aluminum foil or film before cooling.

Treatment Summary

Hot chili (190°F [87°C]) was cooled to 41°F (5°C) using six treatments and three different types of cooling equipment (walk-in refrigerator, blast chiller, and chill stick and walk-in refrigerator):

1. Chili cooled in 12 x 10 x 2 ½" pan, in a walk-in refrigerator
2. Chili cooled in 12 x 10 x 4" pan, in a walk-in refrigerator
3. Chili cooled in 3 gallon stainless steel stockpot, in a walk-in refrigerator
4. Chili cooled in 12 x 10 x 2 ½" pan, in a blast chiller
5. Chili cooled in 12 x 10 x 4" pan, in a blast chiller
6. Chili cooled in 3 gallon stainless steel stockpot, using a chill stick (in a walk-in refrigerator)

Each of the six treatments was replicated three times.

CHAPTER 4. MANUSCRIPTS

This chapter is comprised of two manuscripts presenting results from the two research components of the thesis. The first publication uses preparation guidelines based upon the ASM Style Manual, 2nd ed., 1991, published by the *American Society for Microbiology.* The second manuscript uses preparation guidelines based upon the American Psychological Association (APA) style.

The first manuscript presents research conducted to explore the behavior of *Clostridium perfringens* in turkey using four different retail foodservice cooling methods, and was prepared for submission to the *Journal of Food Protection.* Turkeys were injected with *Clostridium perfringens* spores, cooked, and cooled. Data recording thermometers were used to track temperatures over time during the cooling process. Analyses were conducted to determine relationships between time, temperature, and behavior of the pathogen when retail foodservice cooling methods were used.

The second manuscript presents research related to cooling chili in a retail foodservice establishment, and was prepared for submission to *The Journal of Child Nutrition & Management.* Chili was cooled using six different retail foodservice cooling methods. Data recording thermometers were used to track temperatures over time during the cooling process. Analyses were done to determine the effectiveness of each cooling method, based upon length of cooling times.

INFLUENCE OF FOUR RETAIL FOODSERVICE COOLING METHODS ON THE BEHAVIOR OF *Clostridium perfringens* ATCC 10388 IN TURKEY ROASTS FOLLOWING HEATING TO AN INTERNAL TEMPERATURE OF 74°C

A paper to be submitted for publication in the

Journal of Food Protection

David Olds[1], Aubrey Mendonca[2], Jeannie Sneed[3], and Bledar Bisha[4]

ABSTRACT

The influence of four foodservice cooling methods on germination and growth of *Clostridium perfringens* ATCC 10388 spores in cooked, ready-to-eat turkey roasts was evaluated. Raw whole turkey roasts (~11.25 lbs each) were inoculated with *C. perfringens* spores, vacuum packaged, and heated to an internal temperature of 74°C. The cooked roasts were cooled using four different methods: 1) roasts cut in 4 quarters and cooled in a walk-in refrigerator at 4°C; 2) whole turkey roast cooled in a blast chiller; 3) whole roast loosely wrapped and cooled in a walk-in refrigerator; 4) three whole turkey roasts wrapped together and cooled in a walk-in refrigerator. After the roasts reached an internal temperature of 5°C they

[1] Master of Science candidate, Foodservice and Lodging Management, Iowa State University
[2] Associate Professor, Food Science and Human Nutrition, Iowa State University
[3] Professor, Hotel, Restaurant, and Institution Management, Iowa State University
[4] Graduate student, Food Science and Human Nutrition, Iowa State University

were analyzed for growth of *C. perfringens* using Shahidi-Ferguson perfringens (SFP) agar and anaerobic incubation at 37°C.

None of the cooling methods met the amended 2001 FDA Food Code guidelines (reduction of internal food temperature from 57°C to 21°C in 2 hours and from 21°C to 5°C in 4 hours) for safe cooling of potentially hazardous foods. The time taken for roasts (at 57°C) to reach an internal temperature of 21°C ranged from about 2.3 hours (quartered roast in walk-in refrigerator) to 8.7 hours (3 whole roasts wrapped together in walk-in refrigerator). Temperature reduction (from 57°C to 21°C) for whole roasts in the blast chiller and in the walk-in refrigerator was achieved in about 3 and 6.2 hours, respectively. Time taken for roasts (at 21°C) to reach 5°C ranged from 6.3 hours (refrigerated quartered roasts) to 19.5 hours (3 whole roasts wrapped together in refrigerator). No growth of *C. perfringens* occurred in refrigerated quartered roasts or in whole roasts cooled in the blast chiller; however, populations of the pathogen increased by 1.5 log in the refrigerated single whole roast and by 4.0 log increase in three whole roasts wrapped together in the refrigerator. These findings indicate that cooling methods involving use of walk-in refrigerators (4°C) for decreasing the internal temperature of whole cooked turkey roasts (singly or wrapped together) are unsafe and may result in multiplication of *C. perfringens* to dangerous levels.

INTRODUCTION

Clostridium perfringens food poisoning is an ongoing concern in retail foodservice where large volumes of food product are prepared in advance and cooled before reheating for service. Improper cooling of food was identified as the

number one factor that contributed to the occurrence of 1,918 outbreaks of foodborne illness (FBI) in the United States between 1961-1982 (1). Sporadic illness caused by *C. perfringens* is not reportable through passive or active systems, and data reported are only related to outbreaks (9). *C. perfringens* ranked second or third as the cause of confirmed bacterial foodborne illness outbreaks in the United States from 1983-1997 (7). Many cases of foodborne illnesses may go unreported because the ill person did not seek medical care, the health-care provider did not obtain a sample for diagnosis, the laboratory did not perform the necessary diagnostic test, or the illness or laboratory findings are not communicated to public health officials (9).

Meat and poultry products are the most common food sources of *C. perfringens* type A food poisoning (7). In the *U.S. General Accounting Office (GAO) Report to Congressional Requestors*, state health officials in 32 states were surveyed using a web-based survey instrument regarding 97 outbreaks involving 50 or more individuals between 1990 and 1999 (12). The survey indicated that meat dishes were the number one link to FBI outbreaks, and that *C. perfringens* showed strong epidemiological support as a suspected causative FBI outbreak agent. Improper cooling and food handling methods also were identified as underlying causes of some FBI outbreaks.

Cooking meat products in retail foodservice is normally sufficient to destroy vegetative forms of *C. perfringens.* However, heat resistant spores of *C. perfringens* typically survive the cooking process and are heat-activated, triggering spores to germinate during cooling. Prolonged, improper cooling allows germinated *C.*

perfringens vegetative cells to multiply rapidly, reaching high numbers within slow cooling bulk products such as turkey roasts. In 2003, the Food and Drug Administration (FDA) amended their FDA 2001 Food Code (3, 4), and established a recommendation that all potentially hazardous foods (PHF) be cooled from 57°C to 21°C in two hours and then from 21°C to 5°C in an additional four hours.

Observational studies have shown that retail foodservice procedures might not be meeting these FDA cooling recommendations (5). Henroid and Sneed (6) observed food handling practices of school employees and found that in most school foodservice operations employees were not taking food temperatures. In operations where food temperatures were taken, often temperatures were not documented, employees did not calibrate thermometers, and calibration documentation was non-existent in all schools studied. Henroid and Sneed also observed that six of ten school foodservice operations were not in compliance with FDA standards of cooling potentially hazardous foods (PHF) from 57°C to 21°C in two hours and then from 21°C to 5°C in an additional four hours. Temperatures of foods were not taken or recorded during the cooling process.

In a review of the scientific literature and inspection reports, Snyder (10) found a lack of evidence that health departments were actually performing cooling studies to verify six hour cooling in retail foodservice and that a six hour cooling time was a very difficult standard to meet under typical foodservice refrigeration. The gap between stringent FDA cooling recommendations and verification of cooling standards, coupled with observations of non-compliance of retail foodservice cooling procedures, served as impetus for this study. Further, there is a paucity of

research on time, temperature and growth of *C. perfringens* using commonly observed retail foodservice cooling methods used for raw turkey roasts. Accordingly, the purpose of this research was to evaluate the influence of selected retail foodservice cooling methods on the behavior of *C. perfringens* ATCC 10388 in turkey roasts following heating to an internal temperature of 74°C. Specific objectives included:

1. Develop time and temperature cooling curves for USDA commodity turkey roasts using four cooling methods: quartered turkey roasts cooled in walk-in refrigerator; whole turkey roast cooled in a blast chiller; whole turkey roast loosely wrapped and cooled in walk-in refrigerator; and multiple whole turkey roasts on a sheet pan with an overwrap and cooled in walk-in refrigerator.
2. Compare the growth of *C. perfringens* ATCC 10388 in artificially inoculated turkey roasts cooled using the four cooling methods.

MATERIALS AND METHODS

Test Organism

Clostridium perfringens strains ATCC 10388, NCTC 8238, and NCTC 8239 were obtained from Dr. V. K. Juneja at the USDA Eastern Regional Research Center, Wyndmoor, PA. The organisms were maintained at 4°C in cooked-meat medium (Difco Laboratories, Detroit, MI). *C. perfringens* strains NCTC 8238 and NCTC 8239 were pilot tested and were eliminated from the study after they were determined to be inconsistent in culturability under the conditions of this study. Only *C. perfringens* strain ATCC 10388 was used for this study.

Preparation of Inoculum

Inoculum was prepared using procedures described by Juneja, Call, and Miller (8). To prepare inocula, 0.1 ml of *Clostridium perfringens* strain ATCC 10388 from the stock culture was transferred into 10 ml freshly prepared fluid thioglycollate medium (FTG). The inoculated FTG was heat shocked at 75°C for 20 minutes and incubated at 37°C for 18 hours.

After 18 hours, 1.0 ml of the inoculated FTG was transferred into 10 ml of freshly steamed FTG and incubated at 37°C for 4 hours to produce growth of vegetative cells. After 4 hours, 1.0 ml of the culture in FTG was transferred into Duncan-Strong (DS) sporulation media (2) and incubated at 37°C for 24 hours. DS inoculum was diluted in physiological saline (0.85%). Inoculum was dyed with four drops of a green food grade dye per 10 ml of inoculum immediately before injection into turkey roasts.

Sample Preparation and Inoculation

Forty 5.1 kg USDA commodity boneless symmetrical tube-shaped Jennie-O frozen turkey roasts (more than 93% water with not more than 5.5% dissolved phosphates and aids in mixing, not more than 1.5% salt, not more than 0.5% sodium phosphates) were secured from the USDA's Food, Nutrition, and Consumer Services and kept in cold storage at -17.7°C until required for use. Prior to cooking, roasts were placed in a walk-in refrigerator at 3.9°C for 48 hours until they were completely thawed.

All roasts were inoculated using the same procedure, with a slight modification for treatment 1, as described below. Thawed turkey roasts measuring

14 cm x 40 cm were inoculated by injecting 1 ml of the dyed spore inoculum with a 6-inch (15.24 cm) 20-gauge needle (Fisher Scientific Co., Pittsburgh, PA) in the center of the roast. The inoculum depth of approximately 7 cm per roast was determined by measuring the circumference of the respective roasts and applying the mathematical formula $C = \pi D$ to obtain the radius (½D), which indicated the depth of the center of the roast in which the inoculum was deposited.

Thermal type-K probes (Fisher Scientific Co.) were inserted next to the inoculum at the same depth as the inoculum (approximately 7 cm), in the geometric center of the turkey roast. Each roast was vacuum packed with a Multivac model A 300/51 vacuum packaging machine (Multivac Inc., Kansas City, MO) using Rival VSB2 Seal-a-Meal® plastic bag material (The Holmes Group Inc., Milford, MA), allowing the type-K probe to remain securely in place, and to facilitate cooking without allowing air or liquid to enter. Type-K probes were attached to an Atkins model #37313 data recording digital thermometer (Atkins Technical Inc., Gainesville, FL) set to log temperature data at 10-minute intervals.

Pilot Testing

Cooking, cooling, and enumeration procedures were pilot tested. Two extra roasts were used to supplement treatment #1 (quartered roast) for replications two and three, due to low *C. perfringens* counts observed in pilot testing and in replication one.

Cooking Procedures

Three independent replications of each treatment were performed. Identical procedures were used for each replication. All roasts were prepared and inoculated

according to the previously stated sample preparation and inoculation guidelines and cooked in a steamer at 100°C. All roasts were cooked to the same final internal temperature of 74°C, according to 2001 FDA Food Code (3, 4).

Cooling Procedures

Upon removal from the steamer, roasts were placed under one of the four cooling treatments. In all treatments, each of the four Type-K probes (one for each cooling method) were attached to a separate Atkins data recording thermometer to record cooling temperatures, until turkey roasts reached 5°C.

Summary of Treatments

The cooling treatments for the turkey roasts are summarized in Table 1. For treatment 1, one roast was inoculated using a slightly modified procedure. The 40 cm long roast was marked into four 10 cm quadrants. The roasts were inoculated with 1ml of the dyed *C. perfringens* inoculum at a depth of approximately 7 cm in the center of one of the inner two quadrants. A type-K probe was inserted into the center of the other inner quadrant at a depth of approximately 7 cm. The roast was cooked whole, and then cut into four 10 cm pieces (quadrants). Each section was spread apart, leaving a gap of approximately 7 cm between sections for cooling, and cooled uncovered in a walk-in cooler.

Table 1. Turkey Cooling Treatments

	Number of turkey roasts Per replication	Total
1. One roast quartered, cooled in walk-in cooler	1	3
2. One roast loosely wrapped, cooled in blast chiller	1	3
3. One roast loosely wrapped, cooled in walk-in cooler	1	3
4. Three roasts bagged, cooled in walk-in cooler	3	9
Pilot Test Roasts used:		11
Roasts used to supplement treatment 1:		4
Total Roasts used:		33

For treatment 2, one roast was inoculated, fitted with a type-K probe, cooked, then cooled, loosely covered, in a Servolift Eastern Blast Chiller/Shock Freezer Model #HCM51-20 (Servolift Eastern, Boston, MA). For treatment 3, one roast was inoculated, fitted with a type-K probe, cooked, and cooled, loosely covered, in a walk-in cooler. For treatment 4, three cooked roasts were placed next to one another on a sheet pan and the entire sheet pan was tightly covered with a plastic bag during cooling. The middle roast was considered the roast most likely to take the longest time to cool and was the roast that was inoculated and fitted with a type-K probe.

Microbiological Analysis

After each turkey roast was cooled to 5°C, all inoculated turkey roasts were asceptically cut open using a sterile knife. In an attempt to recover all viable vegetative *C.* perfringens cells from the core of each roast, all dyed cooked meat

was extracted from the surrounding meat. The dyed meat was mixed with 100 ml sterile 0.1% peptone water in a Fisher Brand Filtra Bags (Fisher Scientific Co.) and pummeled for 60 seconds using a Seward Stomacher 400 Lab System Stomacher (Seward Ltd., Thetford, Norfolk, England) on normal speed.

From the meat slurry, 1 ml of filtered fluid was drawn using a sterile pipette, and added to 9 ml of sterile 0.1% peptone water and vortexed to prepare a 1/10 dilution (10^{-1}). This process continued until seven 10-ml tubes of 0.1% peptone water/meat mixture were prepared, from 10^{-1} to 10^{-7} dilutions. From each of these tubes, 1 ml of mixture was transferred into individual Petri dishes and pour-plated with Shahidi-Ferguson-Perfringens (SFP) agar base (Difco Laboratories, Detroit, MI) enriched with a filter-sterilized D-Cycloserine (Sigma Chemical Company, St. Louis, MO) solution. All inoculated SFP D-Cycloserine agar plates were plated in anaerobic jars with Oxoid AnaeroGen™ AN35 sachet bags (Oxoid Ltd., Basingstoke, Hampshire, England) and Oxoid Anaerobic Indicators BR55 (Oxoid Ltd.) and incubated at 37°C for 24 hours. Black colonies on SFP agar plates were counted using a Leica Quebec Darkfield Colony Counter Model #3325 (Wetzlar, Germany).

Data Analysis

Microbiological counts were recorded for all replications. Using Microsoft Excel 2003, time and temperature curves were developed from the downloaded Atkins #37313 data recording thermometer data. Means of all microbiological counts ($\log_{10}$ CFU/roast) and time and temperature data were calculated.

RESULTS AND DISCUSSION

Time and Temperature

Figure 1 shows a graph of the cooling curves (from 57°C to 5°C) for the various cooling treatments. There was great variation in cooling times among the four treatments and none met the amended 2001 Food Code standards (from 57°C to 5°C in 4 hours or 57°C to 21°C in 2 hours and 21°C to 5°C in 4 additional hours).

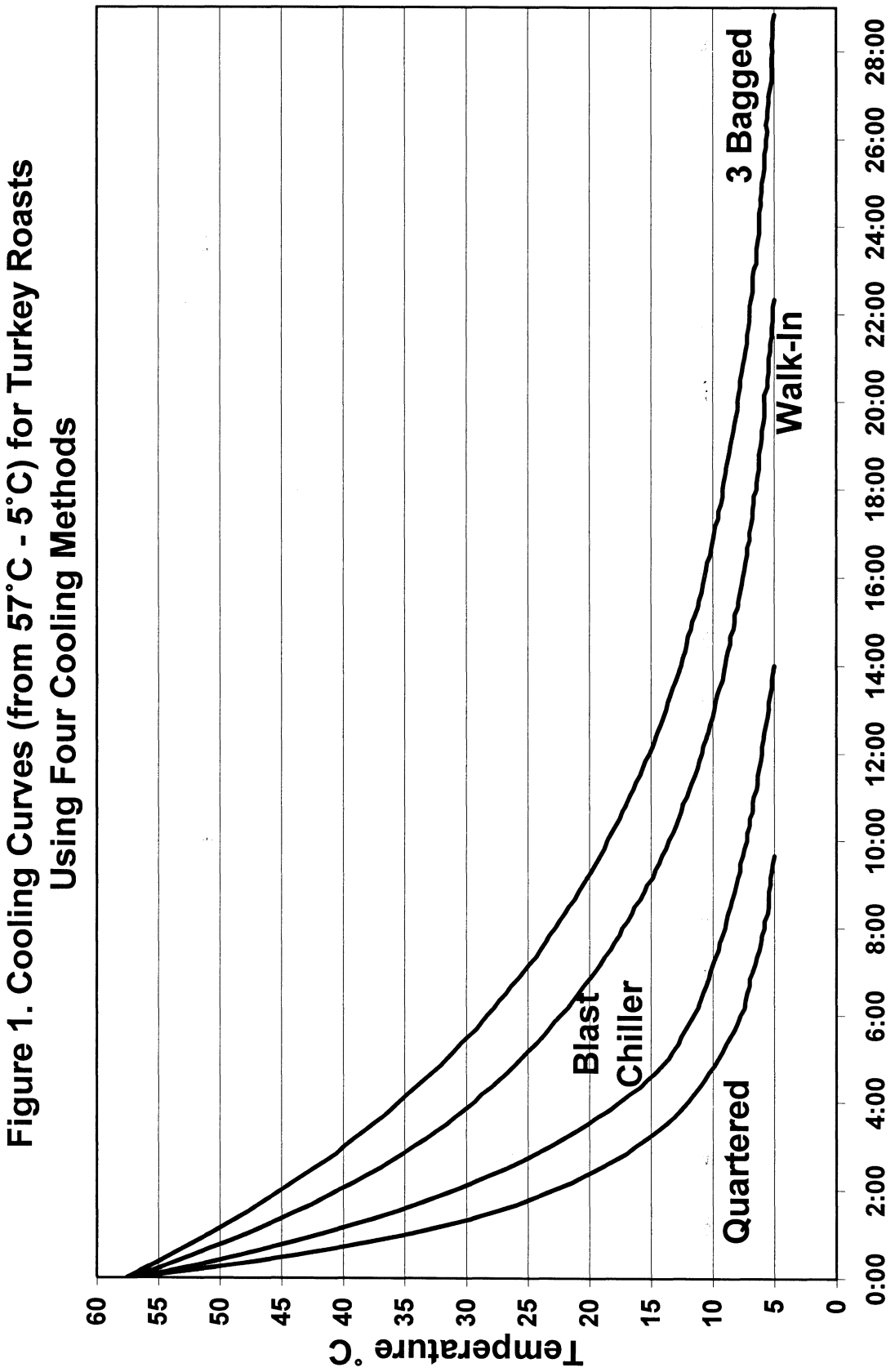
Figure 1. Cooling Curves (from 57°C - 5°C) for Turkey Roasts
Using Four Cooling Methods
Temperature °C
60
55
50
45
40
35
30
25
20
15
10
5
0
0:00
2:00
4:00
6:00
8:00
10:00
12:00
14:00
16:00
18:00
20:00
22:00
24:00
26:00
28:00
Time in Hours
3 Bagged
Walk-In
Blast Chiller
Quartered

Table 2 shows the means of the total cooling times for each treatment from 57°C to 5°C.

Table 2. Mean Time[a] to Cool Turkey Roasts from 57°C to 5°C

Treatment	Rep 1[b]	Rep 2	Rep 3	Mean	± SD
Quartered	8:00	9:40	8:10	8:44	0:51
Blast Chiller	12:30	14:00	10:50	12:26	1:35
Walk-In Refrigerator	16:30	22:20	21:10	20:00	3:05
Three-Bagged	28:00	27:40	28:50	28:10	0:36

[a] hours:minutes
[b] three replications

Table 3 shows the mean cooling times for each treatment from 57°C to 21°C. No cooling treatment achieved the FDA Food Code recommendation of cooling potentially hazardous foods (PHF) from 57°C to 21°C in two hours.

Table 3. Mean Time[a] to Cool Turkey Roasts from 57°C to 21°C

Treatment	Rep 1[b]	Rep 2	Rep 3	Mean	± SD
Quartered	2:10	2:20	2:20	2:16	0:06
Blast Chiller	3:00	3:20	3:00	3:06	0:12
Walk-In Refrigerator	5:50	6:30	6:20	6:13	0:21
Three-Bagged	28:00	27:40	28:50	28:10	0:36

[a] hours:minutes
[b] three replications

Table 4 shows the means of the cooling times for each treatment from 21°C to 5°C. No cooling method achieved the FDA Food Code recommendation of

cooling PHF from 21°C to 5°C in four hours.

Table 4. Mean Time[a] to Cool Turkey Roasts from 21°C to 5°C

Treatment	Rep 1[b]	Rep 2	Rep 3	Mean	± SD
Quartered	5:50	7:20	5:50	6:20	0:52
Blast Chiller	9:30	10:40	7:50	9:20	1:25
Walk-In Refrigerator	10:40	15:50	14:50	13:47	2:24
Three-Bagged	28:00	27:40	28:50	28:10	0:36

[a] hours:minutes
[b] three replications

None of the cooling treatments met the amended 2001 FDA Food Code guidelines. Turkeys were cooled uncovered or loosely covered (except the three-bagged treatment), with the doors of the cooling equipment closed during the entire cooling process, neither of which are common practices in retail foodservice. These practices represented a conservative scenario for the cooling of food, and did not take into account variables in actual foodservice establishments that could potentially increase cooling time, such as opening and closing a refrigerator door or wrapping hot food tightly with aluminum foil or film before cooling.

Of the cooling treatments, it is not surprising to see that the three-bagged treatment took the longest to reach an internal temperature of 21°C or 5°C. By increasing the volume of cooked product around the middle roast and covering with a plastic bag, the insulating effect resulted in a total cooling time of over 28 hours. While this is not a commonly used cooling method, it has been observed in practice

and serves as a worst case scenario for comparison purposes. The single roast cooled in the walk-in refrigerator took less time than the three-bagged treatment, but still took twenty hours to cool. Interestingly, the quartered roast cooled in the walk-in refrigerator took four hours less to cool than the roast in the blast chiller. This dramatically illustrates the insulating effect of food cooled in large quantities, and how volume can trap heat within the center of cooling food.

***C. perfringens* Counts**

Table 5 shows *C. perfringens* profiles of turkey roast treatments in relation to mean cooling times for each treatment. The three-bagged treatment showed a mean **4.00 $\log_{10}$ CFU/roast increase** in viable counts after cooling for a mean time of 28 hours 10 minutes. The walk-in cooling treatment of a whole roast showed a mean **1.50 $\log_{10}$ CFU/roast increase** in viable counts after cooling for a mean time of 20 hours. The blast chiller treatment showed a mean -0.86 $\log_{10}$ CFU/roast decrease in viable counts after cooling for a mean time of 12 hours 26 minutes. Finally, the quartered treatment showed a mean -2.7 $\log_{10}$ CFU/roast decrease in viable counts after cooling for a mean time of 8 hours 44 minutes. Further research is needed to elucidate this result.

It was not surprising that the three-bagged treatment had high microbial counts, due to the extended cooling time, which provided ample opportunity for *C. perfringens* vegetative cells to grow and multiply. The mean 4.00 $\log_{10}$ CFU/roast increase in viable microbial counts far exceeds the USDA/FSIS safe cooling standard criteria (11), allowing no more than a 1 $\log_{10}$ increase between 48.9°C (120°F) and 12.8°C (55°F) within 6 hours as a measure of prevention of the

occurrence of potential *C. perfringens* outbreaks. The whole roast walk-in treatment, which resulted in a mean increase of 1.50 $\log_{10}$ CFU/roast in microbial counts also violated the FSIS/USDA recommendation.

The blast chiller treatment showed a mean decrease of -0.87 $\log_{10}$ CFU/roast in microbial counts, showing that the decreased cooling time actually prevented vegetative *C. perfringens* cells from multiplying. The quartered treatment showed the largest mean decrease of -2.7 $\log_{10}$ CFU/roast, a remarkable decrease in *C. perfringens vegetative* cells. The quartered roast treatment showed an even further reduction than the blast chiller, and with a shorter cooling time, prevented more *C. perfringens* from multiplying than any other treatment.

Table 5. *C. perfringens* Counts (log_{10} CFU/roast) in Turkey

Treatment[a]	Time from 57°C to 5°C In hrs:mins	Initial Spore Count of log_{10} CFU in roasts before cooking	Viable Counts of log_{10} CFU in roasts following cooling	Change in counts of log_{10} CFU in roasts following cooling
Quartered, Walk-In				
	8:44	4.37	≥1.67	≥-2.70
	:51[b]	0.50[b]	≤1.53[b]	1.38[b]
Whole, Blast Chiller				
	12:26	4.23	3.37	-0.86
	1:35[b]	0.63[b]	0.15[b]	0.77[b]
Whole, Walk-In				
	20:00	4.23	5.73	1.50
	3:05[b]	0.63[b]	0.12[b]	0.70[b]
Three-Bagged, Walk-In				
	28:10	4.23	8.23	4.00
	0:36[b]	0.63[b]	0.36[b]	0.61[b]

Note: None of the cooling treatments met the amended 2001 FDA Food Code guidelines.
[a] three replications
[b] standard deviation

CONCLUSIONS

This study simulated actual foodservice cooling methods observed in the foodservice industry. Measurements of time, temperature and growth of *C. perfringens* during cooling of turkey roasts provided data that were compared with

cooling recommendation of the amended 2001 FDA Food Code standards. Significant findings showed that time standards were not met, and typical cooling methods, based on measurements of cooling time and temperature, are unacceptable.

The most significant finding of the study was that two cooling treatments (whole roast cooled in walk-in, three-bagged roasts cooled in walk-in) showed an increase in *C. perfringens* counts, high enough to cause FBI. The other two cooling treatments (quartered roast cooled in walk-in, whole roast cooled in blast chiller) showed a decrease in *C. perfringens* counts, enough to prevent FBI. However, none of the cooling methods met amended 2001 FDA Food code guidelines for cooling PHF from 57°C to 5°C in 4 hours or 57°C to 21°C in 2 hours and 21°C to 5°C in 4 additional hours. Furthermore, none of the cooling methods met USDA/FSIS guidelines (11) for cooling from 48.9°C (120°F) to 12.8°C (55°F) within 6 hours, but yet the quartered roast treatment (mean time of 8:44 to cool) and the blast chilled roast treatment (mean time of 12:26 to cool) both showed log reductions, instead of the presumed ≥1 $\log_{10}$ increase. This may indicate that the established FDA Food Code and FSIS/USDA cooling guidelines are too stringent for foodservice operations.

Methods typically used for cooling whole turkey roasts are unacceptable using normal refrigeration methods. If foodservice operations are unable to invest in a blast chiller, a fairly expensive piece of equipment, then whole cooked turkey roasts should be cut into quarters before cooling in a walk-in refrigerator. Educating foodservice professionals about cooling methods helps convey the value of safe

food and what is needed to keep food safe. Foodservice professionals, in turn, train their employees on an ongoing basis to properly cool food to avoid an outbreak of *C. perfringens.*

More studies are needed on typical cooling methods with different products. Because the FDA Food Code cooling methods cover all potentially hazardous foods (PHF), it is reasonable to suggest that different products may show similar changes in *C. perfringens* counts. In the future, similar inoculation methods with similar cooling treatments could be used to extend the understanding of cooling methods tested in this study.

ACKNOWLEDGMENTS

This research project was supported through an in-kind contribution by USDA. Atkins #37313 data recording thermometers were generously loaned by Sam Beattie, Ph.D., Iowa State University.

REFERENCES

1. Bryan, F. L. 1988. Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. *J. Food Prot.* 51: 663-673.
2. Duncan, C. L., and D. H. Strong. 1968. Improved medium for sporulation of *Clostridium perfringens. Appl. Microbiol.* 16:82-89.
3. FDA/CFSAN FDA 2001 Food Code. 2001. Destruction of organisms - raw animal foods. 2001 Food Code. In *U. S. Department of Health and Human Services Public Health Service Food and Drug Administration 2001 Food Code* (Chap. 3). Available at http://www.cfsan.fda.gov/~dms/fc01-3.html. Accessed June 7, 2004.

4. FDA/CFSAN FDA Supplement to the 2001 Food Code (2003). Food Amendments, Additions, and Deletions. In *U. S. Department of Health and Human Services Public Health Service Food and Drug Administration Supplement to the 2001 Food Code* (Chap. 3). Available at http://www.cfsan.fda.gov/~dms/fc01-sup.html#p1. Accessed May 29, 2004.

5. Food and Drug Administration. 2000. Report of the FDA Retail Food Program Database of Foodborne Illness Risk Factors. Available at: http://vm.cfsan.fda.gov/~acrobat/retrsk.pdf. Accessed March 16, 2004.

6. Henroid, D., and J. Sneed. 2004. Readiness to implement hazard analysis critical control point (HACCP) systems in Iowa schools. *Journal of the American Dietetic Association.* 104:180-185.

7. Heredia, N. L., and R. G. Labbé. 2001. *Clostridium perfringens*, p. 133-141. *In* Ronald G. Labbé and Santos Garcia (eds.), Guide to Foodborne Pathogens. John Wiley & Sons, Inc., New York, New York.

8. Juneja, V. K., J. E. Call, and A. J. Miller 1993. Evaluation of methylxanthines and related compounds to enhance *Clostridium perfringens* sporulation using a modified Duncan and Strong medium. *J. Rapid Methods Automation Microbiol.* 2:203-218.

9. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5: 607-625.

10. Snyder, O. P., and J. A. Labalestra, 2004. Comparison of the bimetallic coil thermometer and thermocouple for validating food cooling. *Food Protection Trends,* 24:423-428.

11. U.S. Department of Agriculture, Food Safety and Inspection Service. 1999. Appendix B – compliance guidelines for cooling heat-treated meat and poultry products (stabilization). Available at: http://www.fsis.usda.gov/OA/fr/95033F-b.htm. Accessed June 12, 2004.

12. U.S. General Accounting Office. 2003. School meal programs: Few outbreaks of foodborne illness reported, but opportunities exist to enhance outbreak data and food safety practices. Available at: http://www.gao.gov/new.items/d03530.pdf. Accessed March 20, 2004.

COOLING RATES OF CHILI USING REFRIGERATOR, BLAST CHILLER, AND CHILL STICK COOLING METHODS

A paper to be submitted for publication in

The Journal of Child Nutrition & Management

David Olds[1] and Jeannie Sneed[2]

ABSTRACT

Purpose/Objectives: The purpose in this study was to determine time and temperature curves for six treatments of chili cooled using three types of cooling equipment utilized in foodservice operations to determine if 2001 FDA Food Code cooling standards were met.

Methods: Chili was cooled using six treatments: (a) chili cooled in 12 x 10 x 2 ½" pan, in a walk-in refrigerator; (b) chili cooled in 12 x 10 x 4" pan, in a walk-in refrigerator; (c) chili cooled in 3 gallon stainless steel stockpot, in a walk-in refrigerator; (d) chili cooled in 12 x 10 x 2 ½" pan, in a blast chiller; (e) chili cooled in 12 x 10 x 4" pan, in a blast chiller; and (f) chili cooled in 3 gallon stainless steel stockpot, using a chill stick, in a walk-in refrigerator. Three replications of each treatment were performed. Mean values of times and temperatures were calculated and cooling curves were graphed for each treatment.

Results: Of three types of cooling equipment used (blast chiller, walk-in refrigerator, and chill stick in refrigerator), only the blast chiller met FDA guidelines

---

[1] Master of Science candidate, Foodservice and Lodging Management, Iowa State University
[2] Professor, Hotel, Restaurant, and Institution Management, Iowa State University

of cooling cooked food from 135°F to 70°F (57°C to 21°C) in two hours, and then from 70°F to 41°F (21°C to 5°C) in four hours. Furthermore, the study found that the use of a chill stick significantly reduced cooling times of chili cooked and then cooled in large volumes.

Applications to Child Nutrition Professionals: Results provide data for Child Nutrition Professionals to make informed choices on food cooling procedures and emphasize the need to evaluate the effectiveness of current cooling methods.

INTRODUCTION

Food safety is a concern for school foodservice directors. Between 1973 and 1999, a reported total of 15,831 foodborne outbreaks effectively caused 447,483 cases of foodborne illness (FBI), 20,119 hospitalizations, and 457 fatalities (U.S. GAO, 2003). Improper cooling of cooked food products has been identified as the number one cause of FBI (Bryan, 1988). Proper cooling of cooked food products has been shown to significantly reduce the chance of outbreaks of foodborne illness, yet cooling often is done incorrectly. Furthermore, equipment for optimal cooling of food frequently is not available in school foodservice operations, and time and temperatures during the cooling process are seldom checked (Henroid & Sneed, 2004).

The *Report of the FDA Retail Food Program Database* (FDA, 2000) stated that 85% of full-service restaurants were not in compliance with FDA standards for cooling potentially hazardous foods (PHF) from 140°F to 70°F (60°C to 21°C) in two hours and then from 70°F to 41°F (21°C to 5°C) in an additional four hours. The FDA report stated that there were inadequate cooling observations to make conclusions

about schools and other institutional settings. However, in a study to evaluate readiness for implementation of Hazard Analysis Critical Control Point (HACCP) systems in schools, Henroid and Sneed (2004) observed that in 10 of 40 school foodservice operations in which cooling was observed, six were not in compliance with FDA standards (FDA/CFSAN, 2001) of cooling potentially hazardous foods (PHF) from 140°F (60°C) to 70°F (21°C) in two hours and then from 70°F (21°C) to 41°F (5°C) in four more hours. Of these non-compliant foodservice operations, Henroid and Sneed observed several cooling practices that were potential food safety hazards.

Some microorganisms, such as *Clostridium perfringens,* can survive cooking and thrive in environments of prolonged cooling, causing serious foodborne illness (Heredia & Labbé, 2001). During cooking, protective heat-resistant spores allow *C. perfringens* to survive high levels of heat that would kill most organisms. During cooling, *C. perfringens* multiply rapidly in the temperature danger zone, which can produce the high microbial levels encountered in food poisoning. After ingesting high levels of *C. perfringens* in improperly cooled food, humans may experience abdominal cramps, bloody diarrhea, and rare cases, even die from *C. perfringens* enterotoxin poisoning (Heredia & Labbé, 2001).

The purpose of this study was to develop time and temperature cooling curves for six treatments of chili cooled using three different types of cooling equipment: a walk-in refrigerator; a blast chiller; and a chill stick. This study provided insights into addressing factors involved in cooling-related FBI outbreaks, and produced results that could: (a) develop educational recommendations for

improving the practices of food handlers; (b) help update and/or verify effective cooling methods for foodservice operations; and (c) provide data for continuing research on foodservice cooling methods.

MATERIALS AND METHODS

Sample and Sample Preparation

Chili con carne with beans was prepared using a recipe from *USDA Recipes for Child Nutrition Programs* obtained from the National Food Service Management Institute (NFSMI, 1999). USDA commodity ground beef was secured for the study.

Cooking Procedures

Chili was prepared in a quantity food production laboratory at Iowa State University, using a 15-gallon Cleveland tilting skillet. All recipe ingredients and cooking procedures were followed exactly, under carefully controlled laboratory conditions.

Cooling Procedures

After cooking, the chili was transferred while still hot (190°F [87°C]) into three different sized containers for cooling. Three quarts of hot chili were placed into a 12"x10"x 2 ½" stainless steel pan. Five quarts of hot chili were placed into a 12"x10"x4" stainless steel pan. Three gallons of hot chili were placed into a stainless steel stockpot. The same stainless steel containers were used for all three replications of the experiment.

A K-type probe was placed in the geometric center of the chili to measure the hottest area of the container during cooling. The Type-K probe was attached to an Atkins model #37313 data recording thermometer programmed to read

temperatures at 10-minute intervals. Chili was cooled uncovered, with the doors to the cooling equipment closed during the entire cooling process.

Treatments

Hot chili was cooled to 41°F (5°C) using six treatments and three different types of cooling equipment (walk-in refrigerator, blast chiller, and chill stick and walk-in refrigerator):

1. Chili cooled in 12 x 10 x 2 ½" pan, in a walk-in refrigerator
2. Chili cooled in 12 x 10 x 4" pan, in a walk-in refrigerator
3. Chili cooled in 3 gallon stainless steel stockpot, in a walk-in refrigerator
4. Chili cooled in 12 x 10 x 2 ½" pan, in a blast chiller
5. Chili cooled in 12 x 10 x 4" pan, in a blast chiller
6. Chili cooled in 3 gallon stainless steel stockpot, using a chill stick (in a walk-in refrigerator)

Each of the six treatments was replicated three times.

RESULTS AND DISCUSSION

As shown in Figure 1, the chili cooling time varied greatly among the six treatments. Of the six treatments, Table 1 shows that only the 2-inch and 4-inch blast-chilled pans of chili met the amended 2001 FDA Food Code cooling guidelines for potentially hazardous foods [cooling from 135°F (57°C) to 70°F (21°C) in two hours and then from 70°F (21°C) to 41°F (5°C) in an additional four hours].

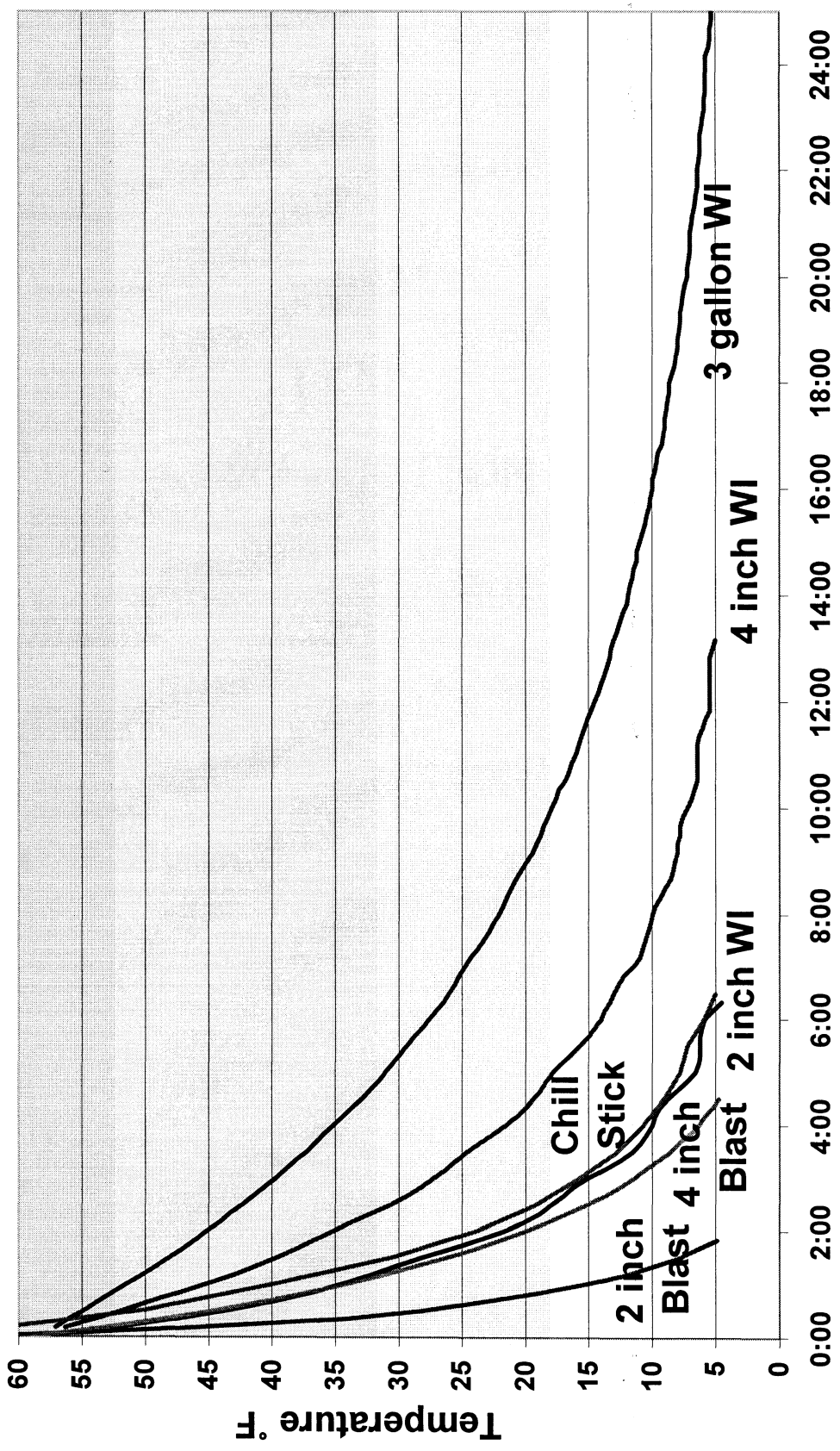

Figure 2. Cooling Curves (from 135˚F to 41˚F) for Chili Using Six Cooling Methods

Table 1. Mean Time[a] for Cooling Three Replications of Chili from 135°F to 41°F Using Six Cooling Methods

Treatment	1	2	3	Mean	± SD
2-inch Walk-in	6:20	7:10	8:30	7:20	1:06
4-inch Walk-in	13:10	10:40	10:50	11:33	1:24
3 gallon Walk-in	26:00	22:40	24:10	24:17	1:40
2 inch Blast Chiller	1:50	2:00	2:00	1:57	0:06
4 inch Blast Chiller	4:30	3:50	4:20	4:13	0:20
3 gallon Chill Stick	6:10	6:10	6:10	6:10	0:00

NOTE: FDA requires that cooked food be cooled from 135°F to 41°F in six hours (135°F to 70°F in two hours and then from 70°F to 41°F in an additional four hours).

[a] hours:minutes

The three walk-in cooling treatments showed the greatest deviation from of the amended 2001 FDA Food Code guidelines (FDA/CFSAN, 2003), whereas the other three treatments met or came very close to meeting guidelines. The three gallon stockpot of chili took over 24 hours to cool in the walk-in refrigerator, but with the addition of a chill stick, it reduced cooling time by approximately 18 hours.

Tables 2 and 3 show that although the chill stick took two hours and ten minutes to cool from 135°F (57°C) to 70°F (21°C), a ten minute violation of the amended 2001 FDA Food Code, it passed the four hour standard by cooling from 70°F (21°C) to 41°F (5°C) in exactly four hours. Although the chill stick narrowly missed meeting FDA recommendations, it showed a significant reduction in cooling time, and might meet recommendations if it were used in a smaller volume of chili.

Table 2. Mean Time[a] for Cooling Three Replications of Chili from 135°F to 70°F Using Six Cooling Methods

Treatment	1	2	3	Mean	± SD
2-inch Walk-in	2:00	2:40	2:30	2:23	0:21
4-inch Walk-in	4:00	3:40	4:00	3:53	0:12
3 gallon Walk-in	8:20	7:40	8:00	8:00	0:20
2 inch Blast Chiller	0:40	0:40	0:40	0:40	0:00
4 inch Blast Chiller	1:50	1:30	1:50	1:43	0:12
3 gallon Chill Stick	2:10	2:10	2:10	2:10	0:00

NOTE: FDA requires that cooked food be cooled from 135°F to 70°F in two hours.

[a] hours:minutes

Table 3. Mean Time[a] for Cooling Three Replications of Chili from 70°F to 41°F Using Six Cooling Methods

Treatment	1	2	3	Mean	± SD
2-inch Walk-in	4:20	4:30	6:00	4:57	0:55
4-inch Walk-in	9:10	7:00	6:50	7:40	1:18
3 gallon Walk-in	17:40	15:00	16:10	16:17	1:20
2 inch Blast Chiller	1:10	1:20	1:20	1:17	0:06
4 inch Blast Chiller	2:40	2:20	2:30	2:30	0:10
3 gallon Chill Stick	4:00	4:00	4:00	4:00	0:00

NOTE: FDA requires that cooked food be cooled from 70°F to 41°F in four hours.

[a] hours:minutes

CONCLUSIONS AND APPLICATIONS

Results of this study show that cooling methods most often used in schools are ineffective in meeting the amended 2001 Food Code cooling standards. Blast cooling methods were the only ones that met all standards. Further, practices in this study represented a "best case" scenario for the cooling of chili, and did not take into account variables in actual foodservice establishments that could potentially increase cooling time, such as opening and closing a refrigerator door or wrapping hot food tightly with aluminum foil or film before cooling.

These results suggest several applications for school foodservice:

- The effectiveness of currently used cooling methods should be determined and documented. If the Food Code standards are not met, changes are needed to meet the standards.
- Blast chillers should be considered when foodservice directors are building new kitchen or when monies are available to upgrade equipment.
- Inexpensive equipment such as chill sticks can be used to decrease the cooling times for liquid products such as chili, soups, etc.
- Standard operating procedures for cooling various type of products need to be developed for each school.
- Employee training on appropriate cooling methods is needed.

ACKNOWLEDGMENTS

This research project was supported through an in-kind contribution of USDA ground beef. Atkins data recording thermometers were generously loaned by Sam Beattie, Ph.D., Iowa State University.

REFERENCES

Bryan, F. L. (1988). Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. *Journal of Food Protection, 51*, 663-673.

FDA/CFSAN FDA 2001 Food Code (2001). Destruction of Organisms - Raw Animal Foods. 2001 Food Code. In *U. S. Department of Health and Human Services Public Health Service Food and Drug Administration 2001 Food Code* (Chap. 3). Retrieved May 29, 2004, from http://www.cfsan.fda.gov/~dms/fc01-3.html

FDA/CFSAN FDA Supplement to the 2001 Food Code (2003). Food Amendments, Additions, and Deletions. In *U. S. Department of Health and Human Services Public Health Service Food and Drug Administration Supplement to the 2001 Food Code* (Chap. 3). Retrieved May 29, 2004, from http://www.cfsan.fda.gov/~dms/fc01-sup.html#p1

Food and Drug Administration. (2000). Report of the FDA retail food program database of foodborne illness risk factors. Available: http://vm.cfsan.fda.gov/~acrobat/retrsk.pdf. Accessed May 31, 2004.

Henroid, D., & Sneed, J. (2004). Readiness to implement hazard analysis critical control point (HACCP) systems in Iowa schools. *Journal of the American Dietetic Association, 104,* 180-185.

Heredia, N. L., & Labbé, R. G. (2001). *Clostridium perfringens*. In Ronald G. Labbé and Santos Garcia (Eds.), *Guide to Foodborne Pathogens* (pp. 133-141). New York: John Wiley & Sons, Inc.

National Food Service Management Institute. (1999). Chili con carne with beans. In USDA Recipes for Child Nutrition Programs. Available: http://www.nfsmi.org/Information/recipes/d_20.pdf. Accessed May 31, 2004.

U.S. General Accounting Office. (2003). School meal programs: Few outbreaks of foodborne illness reported, but opportunities exist to enhance outbreak data and food safety practices. Available: http://www.gao.gov/new.items/d03530.pdf. Accessed May 29, 2004.

CHAPTER 5. GENERAL CONCLUSIONS

Summary and Conclusions

The purpose of this research was to ascertain the effectiveness of applying commonly observed retail foodservice cooling methods to cool selected meat products. This was accomplished by measuring temperature in turkey roasts and chili over time while cooling and comparing these measurements to established FDA Food Code standards of food preparation. The growth of *C. perfringens* in turkey roasts was measured when the product was cooled to 41°F (5°C). This research included two experiments. The first experiment focused primarily upon the microbiological behavior of *C. perfringens* in turkey roasts after applying selected cooling methods within a laboratory environment. The second experiment measured time and temperature in chili, using several cooling treatments within a retail foodservice operation.

The turkey cooling experiment simulated actual foodservice cooling methods observed in the foodservice industry. Measurements of time, temperature, and growth of *C. perfringens* provided data with which comparisons could be made to amended 2001 FDA Food Code standards. Significant findings of the study showed that time standards were not being met, and that typical foodservice cooling methods were unacceptable.

The most significant finding of the turkey cooling study was that two cooling treatments (whole roast cooled in walk-in, three-bagged roasts cooled in walk-in) showed an increase in *C. perfringens* counts high enough to cause FBI. The other two cooling treatments (quartered roast cooled in walk-in, whole roast cooled in

blast chiller) showed a decrease in *C. perfringens* counts, enough to prevent FBI. However, none of the cooling methods met amended 2001 FDA Food code guidelines for cooling PHF from 57°C to 5°C in 4 hours or 57°C to 21°C in 2 hours and 21°C to 5°C in 4 additional hours. Furthermore, none of the cooling methods met USDA/FSIS guidelines for cooling from 48.9°C (120°F) to 12.8°C (55°F) within 6 hours, but yet the quartered roast treatment (mean time of 8:44 to cool) and the blast chilled roast treatment (mean time of 12:26 to cool) showed mean 2.7 and 0.86 $\log_{10}$ reductions in *C. perfringens*, respectively, instead of the presumed ≤1 $\log_{10}$ increase. This may indicate that the established FDA Food Code and FSIS/USDA cooling guidelines are too stringent for foodservice operations.

Typical foodservice refrigeration methods used for cooling whole turkey roasts are unacceptable. If foodservice operations are unable to invest in a blast chiller, then whole cooked turkey roasts should be cut into quarters before cooling under refrigeration.

Blast-chilled chili in 2-inch and 4-inch pans met food cooling guidelines, but chili cooled using the same sized pans under typical foodservice refrigeration did not. Chili cooled in 3-gallon increments under typical foodservice refrigeration showed a significant violation of food cooling guidelines, taking approximately 24 hours to cool from 135°F to 41°F. With the addition of a chill stick, the same 3-gallon container of chili cooled under typical foodservice refrigeration showed a 75% reduction in cooling time to approximately 6 hours. These results suggest several applications for foodservice:

- The effectiveness of currently used cooling methods should be determined and documented. If the Food Code standards are not met, changes are needed to meet the standards.
- Standard operating procedures for cooling various types of products need to be developed based upon the effectiveness of meeting Food Code standards.
- Blast chillers should be considered when building new kitchens or when monies are available to upgrade equipment.
- Inexpensive equipment such as chill sticks can be used to decrease the cooling times for liquid products such as chili, soups, etc.
- Education about cooling methods helps to convey the importance and value of safe food and what is needed to keep food safe. Foodservice professionals need to provide continuing training for employees in proper cooling methods and explain to employees the reasons why cooling is important.
- More studies are needed using typical and atypical cooling methods with different food products to extend the understanding of cooling methods tested in this study.

Limitations of the Study

Only two meat products (turkey roasts and ground beef) were studied. For safety and validity reasons, the research was conducted in a microbiological laboratory. Although the simulation of actual foodservice cooling procedures occurred, the exact duplication of a retail foodservice operation within a laboratory

was not feasible. Furthermore, variables related to food handlers, such as handwashing frequency and timing, were controlled in the laboratory setting and may not have replicated actual practices.

Recommendations for Future Study

More studies are needed on typical cooling methods with different products. Because the FDA Food Code cooling methods cover all potentially hazardous foods (PHF), it is reasonable to suggest that different products may show similar changes in *C. perfringens* counts. In the future, similar inoculation methods with similar cooling treatments could be used to extend the understanding of cooling methods tested in this study.

Further research should be conducted with the quartered roast and blast chilled turkey treatments to elucidate reasons for the slight decline in viability of *C. perfringens* under these conditions. Microbiological data should be collected for future replications of chili cooling to provide comparisons of *C. perfringens* counts with time and temperature curves using foodservice cooling methods.

APPENDIX A. STANDARD OPERATING PROCEDURES

STANDARD OPERATING PROCEDURE #1

Title: Procedure #1: Fluid Thioglycollate Medium (FTM) preparation (for enumeration of *Clostridium perfringens* vegetative cells)
Dept: Food Science and Human Nutrition
Lab: FSB 3378
Supervisor: Dr. Aubrey Mendonca

Procedure Overview: To make twenty-five 25mm x 125mm culture tubes each containing 10ml FTM.

Materials and Equipment

- 7.45g Thioglycollate Medium (FTG) T-9032 500g, (Sigma Chemical Co., St. Louis, MO)
- 500ml flask with magnetic stir bar
- 250ml graduated cylinder
- 250ml dH_2O
- Thermolyne Type 1000 Stir Plate (Barnstead/Thermolyne, Dubuque, IA)
- Pipet-aid electric pipette pump, Drummond Scientific Co. (Broomall, PA)
- 25ml Fisherbrand 25 ml pipette Cat.# 13-678-11 (Fisher Scientific Co., Pittsburgh, PA)
- Twenty-five 25mm x 125mm culture tubes
- Twenty-five rubber stoppers for culture tubes
- Test tube rack
- Primus PSS5000 Steam Sterilizer (Primus Sterilizer Co. Inc., Omaha NE)

Safety Precautions:

- Gloves
- Freshly prepared Fluid Thioglycollate Medium (FTM) has a stench

Procedure:

1. Add 7.45 g Thioglycollate Medium (FTG) to 250 ml dH_2O in 500ml flask with magnetic stir bar to make Fluid Thioglycollate Medium (FTM).
2. Mix thoroughly and bring Fluid Thioglycollate Medium (FTM) to a boil.
3. Using Pipet-aid electric pipette pump and 25 ml pipette, transfer 10 ml of FTM into 25mm x 125mm culture tube. Repeat until all twenty-five culture tubes are filled.
4. Loosely cap culture tubes with rubber stoppers.
5. Autoclave on setting #1 (<4L of media).
6. Use freshly prepared FTM promptly or store under refrigeration at 4°C until use.
7. Finished FTM will turn from a yellow to a pink color in the presence of oxygen.

8. If using from refrigerated storage, freshly steam FTM until no pink color remains to ensure reduction of oxygen before use (e.g. enumeration of *Clostridium perfringens* vegetative cells).

STANDARD OPERATING PROCEDURE #2

Title: Procedure #2: Enumeration of *Clostridium perfringens* vegetative cells from selected stock spore cultures (ATCC 10388, NCTC 8238, or NCTC 8239)
Dept: Food Science and Human Nutrition
Lab: FSB 3378
Supervisor: Dr. Aubrey Mendonca

Procedure Overview: To enumerate C. perfringens vegetative cells from selected stock spore cultures.

Materials and Equipment

- 10 ml Fluid Thioglycollate Medium (FTM) prepared in 25mm x 125mm culture tubes [*see S.O.P. – "Procedure #1 - Fluid Thioglycollate Medium (FTM) preparation (for enumeration of *Clostridium perfringens* vegetative cells)"].
- Test tube rack
- Steamer
- *C. perfringens* stock spore culture (ATCC 10388, NCTC 8238, or 8239) stored in cooked meat medium (Difco, Detroit, MI) at 4°C.
- 2.0ml Pipette Pump™ Pipettor #F37897 (Bel-Art Products, Pequannock, NJ)
- 1.0ml pipette Cat.#13-678-11B (Fisher Scientific Co., Pittsburgh, PA)
- Nitrogen atmosphere evacuation system
- Pliers for heat-fixing 25mm x 125mm culture tube rubber stopper [*see S.O.P. – "Procedure #1 -Fluid Thioglycollate Medium (FTM) preparation (for enumeration of *Clostridium perfringens* vegetative cells)"].
- Bunsen burner, FisherBrand #1201-21 (Fisher Scientific Co.)
- Parafilm "M" Laboratory Film #PM-996 (Pechiney Plastic Film Packaging, Chicago, IL)
- Pipette bag rack
- Rubber bands
- Water bath, IsoTemp Model #10135 (Fisher Scientific Co.)
- Low-temperature incubator Model #815 (Precision Scientific Co., Chicago, IL)

Safety Precautions:

- Gloves
- Freshly prepared Fluid Thioglycollate Medium (FTM) has a stench.
- Flaming rubber stoppers produces a stench.
- FTM inoculated with *C. perfringens* vegetative cells will vigorously bubble with gases – **do not attempt to vortex FTM inocula** in 25mm x 125mm culture tubes with rubber stoppers, even if they are sealed with plastic. Vortexing will agitate the gases and the increased pressure will blow the stopper out of the test tube along with the FTM inocula.

Procedure:

1. Freshly steam Fluid Thioglycollate Medium (FTM) so that no pink color remains.
2. Using 1.0ml pipette, transfer 0.1ml of selected *C. perfringens* stock spore culture (ATCC 10388, NCTC 8238, or 8239) into 10ml freshly steamed FTM in 25mm x 125mm culture tube.
3. Use pipette to mix culture into FTM, **do not vortex** (see "Safety Precautions").
4. Purge the head space in 25mm x 125mm culture tube with nitrogen.
5. Using pliers, flame rubber stopper with Bunsen burner until flaming and smoking.
6. Cap culture tube tightly with flaming/smoking rubber stopper.
7. Seal tightly with Parafilm laboratory film.
8. Attach tightly-sealed 25mm x 125mm culture tube to pipette bag stand vertically with rubber bands.
9. Heat shock pipette bag stand (with attached 25mm x 125mm culture tube containing 10ml FTM *C. perfringens* spore inocula) in water bath at 75°C for 20 minutes.
10. Be sure that the top of the inocula level in the culture tube is slightly submerged under the water level in the water bath.
11. Remove culture tube from water bath and incubate heat-shocked FTM *C. perfringens* spore inocula at 37°C for 18 hours.
12. After 18 hours, transfer 1.0ml of FTM *C. perfringens* spore inocula to another 25mm x 125mm culture tube containing 10ml freshly-steamed FTM.
13. Follow the same procedural steps (3-7) to nitrogen evacuate atmosphere and seal culture tube.
14. Incubate at 37°C for 4 hours.

FTM culture is now enumerated with *Clostridium perfringens* vegetative cells.

STANDARD OPERATING PROCEDURE #3

Title: Procedure #3: Enumeration of *Clostridium perfringens* spores using Duncan-Strong (DS) Sporulation Medium
Dept: Food Science and Human Nutrition
Lab: FSB 3378
Supervisor: Dr. Aubrey Mendonca

Procedure Overview: **To enumerate *C. perfringens* spores from FTM C. *perfringens* vegetative cells using Duncan-Strong (DS) Sporulation Medium.**

Materials and Equipment

- Duncan and Strong (DS) Media, Modified (for *C. perfringens*)
  - 1L Flask with magnetic stir bar
  - 500ml graduated cylinder
  - 500ml dH_2O
  - 7.5 Proteose Peptone No. 3 #211693 (Difco Laboratories, Detroit, MI)
  - 2g Bacto™ Yeast Extract #212750 (Difco Laboratories)
  - 5g Sodium Phosphate ($Na_2HPO_47H_2O$) #S-373 (Fisher Scientific Co., Pittsburgh, PA)
  - 2g Raffinose #R7630 (Sigma Chemical Co., St. Louis, MO)
  - Thermolyne Type 1000 Stir Plate, (Barnstead/Thermolyne, Dubuque, IA)
  - 100ml graduated cylinder
  - Four 250ml flasks
  - Four Caps for 250ml flasks
  - Autoclave
- 2.0ml Pipette Pump™ Pipettor #F37897 (Bel-Art Products, Pequannock, NJ)
- 1.0ml pipette Cat.#13-678-11B (Fisher Scientific Co., Pittsburgh, PA)
- 1 ml FTM culture of enumerated *Clostridium perfringens* vegetative cells [*see S.O.P. (step 15) – "Procedure #2 - Enumeration of *Clostridium perfringens* vegetative cells from selected stock spore cultures (ATCC 10388, NCTC 8238, or NCTC 8239)"].

Safety Precautions:

- Gloves
- FTM culture of enumerated *C. perfringens* vegetative cells will vigorously bubble with gases – **do not attempt to vortex FTM inocula** in 25mm x 125mm culture tubes with rubber stoppers, even if they are sealed with plastic. Vortexing will agitate the gases and the increased pressure will blow the stopper out of the test tube along with the FTM inocula.

Procedure:

1. Make Duncan-Strong (DS) Sporulation Medium, by putting 7.5 proteose peptone, 2g yeast extract, 5g sodium phosphate ($Na_2HPO_47H_2O$), and 2g raffinose into a 1L flask containing 500ml dH_2O and a magnetic stir bar.
2. Place DS Sporulation Medium on Thermolyne Type 1000 Stir Plate, and stir. Turn on heat and bring to a boil.
3. Distribute 100ml of DS Sporulation Medium into each 250ml flask and cap loosely.
4. Autoclave on setting #1 (<4L of media).
5. Allow DS Sporulation Medium to cool. Store at 4°C until use.
6. Transfer 1 ml FTM culture of enumerated *Clostridium perfringens* vegetative cells [*see S.O.P. (step 15) – "Procedure #2 - Enumeration of *Clostridium perfringens* vegetative cells from selected stock spore cultures (ATCC 10388, NCTC 8238, or NCTC 8239)"] into 100ml Duncan-Strong (DS) Sporulation Medium.
7. Incubate DS Sporulation Medium at 37°C for 24 hours.
8. Store DS Sporulation Medium at 4°C until use.

DS Sporulation Medium is now enumerated with *Clostridium perfringens* spores.

STANDARD OPERATING PROCEDURE #4

Title: Procedure #4: Preparation of Shahidi-Ferguson-*perfringens* (SFP) agar base enriched with filter-sterilized D-Cycloserine solution
Dept: Food Science and Human Nutrition
Lab: FSB 3378
Supervisor: Dr. Aubrey Mendonca

Procedure Overview: To make 1L of SFP D-Cycloserine Agar.
Materials and Equipment

- 2L flask with magnetic stir bar
- 1L graduated cylinder
- 1L dH_2O
- 52.2g SFP Agar Base #281110 (Difco Laboratories, Detroit, MI)
- Two 1L flasks
- Thermolyne Type 1000 Stir Plate (Barnstead/Thermolyne, Dubuque, IA)
- IsoTemp Water Bath Model #228 (Fisher Scientific Co., Pittsburgh, PA)
- 100ml flask with stir bar
- 80ml dH_2O
- 0.4g D-Cycloserine C6880-5G (Sigma Chemical Co., St. Louis, MO)
- Millipore Filter System - Glass Filter, Stopper, Clamp, (Millipore, Billerica, MA)
- Millipore Filter .22µm 47mm #GSWP047S0 (Millipore)
- 500ml filter flask
- Vacuum pump #6125 (Nalgene Nunc Intl., Rochester, NY)
- Pipet-aid electric pipette pump, Drummond Scientific Co. (Broomall, PA)
- 25ml Fisherbrand 25 ml pipette Cat.# 13-678-11 (Fisher Scientific Co., Pittsburgh, PA)

Safety Precautions:

- Gloves
- D-Cycloserine is heat-sensitive, do not boil or add to SFP agar that is over 50.0°C.
- Discard SFP D-Cycloserine agar after 4 hours.

Procedure:

1. Add 52.2g SFP Agar Base to a 2L flask containing 1L dH_2O and a stir bar.
2. Cover. Stir well with Thermolyne Type 1000 Stir Plate and bring to a boil.
3. Divide SFP agar into two 1L flasks (500ml in each), both flasks containing stir bars.
4. Cap loosely both 1L flasks of boiled SFP agar
5. Autoclave on setting #1 (<4L of media).
6. Place 1L flasks of SFP agar into IsoTemp Water Bath Model #228 and stabilize temperature at 50.0°C.

7. Place 0.4g D-Cycloserine into 100ml flask containing 80ml dH_2O and a stir bar.
8. Cover and stir D-Cycloserine (no heat) with Thermolyne Type 1000 Stir Plate until solution is well mixed.
9. Filter-sterilize 80ml D-Cycloserine solution using Millipore Filter Sterilization system.
10. Use 25ml Fisherbrand 25 ml pipette and Pipet-aid electric pipette pump to pipette 40ml of filtered D-Cycloserine solution into 500ml of SFP agar stabilized at 50.0°C. Repeat procedure for second flask.
11. Place SFP D-Cycloserine solution on stir plate and mix well. Hold in water bath at 50.0°C. Repeat procedure for second flask.
12. Use SFP D-Cycloserine agar within 4 hours. Discard any leftover SFP D-Cycloserine agar.

STANDARD OPERATING PROCEDURE #5

Title: Procedure #5: Inoculation and preparation of turkey roasts for cooking
Dept: Food Science and Human Nutrition
Lab: FSB 3378
Supervisor: Dr. Aubrey Mendonca

Procedure Overview: To inoculate and prepare turkey roasts for cooking.
Materials and Equipment

- 11.25 pound, 14cm x 40cm USDA commodity issued boneless symmetrical tube-shaped Jennie-O frozen turkey roast (more than 93% water with not more than 5.5% dissolved phosphates and aids in mixing, not more than 1.5% salt, not more than 0.5% sodium phosphates), thawed to 4°C.
- Cloth metric tape measure
- Type-K Beaded Probe #15-077-45 (Fisher Scientific Co., Pittsburgh, PA)
- FisherBrand Autoclave tape #12-0 (Fisher Scientific Co.)
- 5ml DS Sporulation Medium enumerated with *Clostridium perfringens* spores [*see S.O.P. (step 9) – "Procedure #3 - Enumeration of *Clostridium perfringens* spores using Duncan-Strong (DS) Sporulation Medium."]
- 5ml 0.85% dH_2O buffer in 16mm x 125mm culture tube.
- Eyedropper
- Green food coloring (McCormick & Co. Inc., Hunt Valley, MD)
- Vortex Genie 2, Cat#12-812 (Fisher Scientific Co.)
- 3ml BD Luer Lok® syringe (Becton Dickinson & Co., Franklin Lakes, NJ)
- Two 6 inch (15.24 cm) 20 gauge needles, sterile (Fisher Scientific Co.)
- 2.0ml Pipette Pump™ Pipettor #F37897 (Bel-Art Products, Pequannock, NJ)
- 1.0ml pipette Cat.#13-678-11B (Fisher Scientific Co., Pittsburgh, PA)
- Rival VSB2 Seal-a-Meal® plastic bag material
- Hot Glue Gun
- Multivac model A 300/51 Vacuum Packager (Multivac Inc., Kansas City, MO)
- Atkins #37313 Data recording Thermometer (Atkins Technical Inc., Gainesville, FL)
- RS232 Cable to connect thermometer to laptop computer
- Gateway Solo 1100 Laptop computer (Windows XP) with Atkins #37313 data recording thermometer software installed.
- Steamer
- DS1921L-52 Thermochron iButton (Dallas Semiconductor/Maxim, Sunnyvale, California)

Safety Precautions:

- Gloves

Procedure:

1. Measure the circumference of the turkey roast using a cloth tape measure,.
2. Apply the mathematical formula $C = \pi D$ to obtain the radius (½D), which indicates the depth of the center of the roast in which to install thermal Type-K Beaded Probe and to inoculate.
3. Mark all 6-inch needles to the length of the radius.
4. Mark thermal Type-K Beaded Probe to the length of the radius.
5. Make a hole at radius depth near the center of the roast with one of the 6-inch needles. **Do not reuse needle.**
6. Insert Type-K Beaded Probe to radius depth.
7. Secure Type-K Beaded Probe with autoclave tape.
8. Transfer 5ml of DS Sporulation Medium enumerated with *Clostridium perfringens spores* [*see S.O.P. (step 9) – "Procedure #3 - Enumeration of *Clostridium perfringens* spores using Duncan-Strong (DS) Sporulation Medium."] into 5ml 0.85% dH_2O buffer in 16mm x 125mm culture tube to make *C. perfringens* spore inoculum.
9. Add 4 drops green food coloring, using eyedropper.
10. Cap culture tube and vortex thoroughly.
11. Preheat Hot Glue Gun
12. Attach another 6-inch needle to 3ml BD Luer Lok® syringe. Remove plunger and set aside, upside-down, keeping it sterile.
13. Insert needle to marked radius depth near center of turkey roast and the Type-K Beaded Probe .
14. Pipette 1ml of green-dyed *C. perfringens* spore inoculum into syringe.
15. Replace plunger and firmly inject green-dyed *C. perfringens* spore inoculum into roast.
16. Wait two minutes and quickly pull needle from roast.
17. Seal hole immediately in plastic with a dollop of hot glue.
18. Allow to cool.
19. Package turkey roast in Rival VSB2 Seal-a-Meal® plastic bag material, sealed on three sides, with Type-K Beaded Probe wire exiting the opening.
20. Apply glue using glue gun to Type-K Beaded Probe wire and bag exiting the roast near the "sealing bar" on the vacuum packaging machine.
21. Vacuum-pack turkey roast using Multivac model A 300/51 vacuum packaging machine.
22. Pre-program Atkins #37313 data recording thermometer using Atkins #37313 software installed on a Gateway Solo 1100 laptop computer running Windows XP by means of a RS232 connecter cable.
23. Attach Type-K Beaded Probe to Atkins #37313 data recording thermometer.
24. Place roast in steamer
25. Steam turkey at 100°C until internal temperature of 74°C.
26. Remove to selected cooling treatment and cool to 4°C.
27. Use DS1921L-52 Thermochron iButton to track ambient air temperature of cooling unit.

28. Heat shock 5ml of green-dyed *C. perfringens* spore inoculum at 75°C for 20 minutes, perform 1/10 dilutions and pour plate 0.1ml of each dilution with SFP D-Cycloserine Agar [*see Procedure #4 – Preparation of Shahidi-Ferguson-*Perfringens* (SFP) agar base enriched with filter-sterilized D-Cycloserine solution]. Incubate plates in an anaerobic jar with a Oxoid AnaeroGen™ AN35 sachet bag at 37° for 24 hours [*see Procedure #6 – "Procedure #6 – Analysis of turkey roasts inoculated with *C. perfringens* spores after cooking and cooling."].

STANDARD OPERATING PROCEDURE #6

Title: Procedure #6: Analysis of turkey roasts inoculated with C. perfringens spores after cooking and cooling
Dept: Food Science and Human Nutrition
Lab: FSB 3378
Supervisor: Dr. Aubrey Mendonca

Procedure Overview: To analyze turkey roasts inoculated with C. perfringens spores after cooking and cooling.

Materials and Equipment

- 11.25 pound, 14cm x 40cm USDA commodity issued boneless symmetrical tube-shaped Jennie-O turkey roast, inoculated with C. perfringens spores, cooked and cooled
- Atkins #37313 Data recording Thermometer (Atkins Technical Inc., Gainesville, FL)
- RS232 Cable to connect thermometer to laptop computer
- Gateway Solo 1100 Laptop computer (Windows XP) with Atkins #37313 data recording thermometer software installed.
- Sanitized Cutting board
- Aseptic (sterile) carving knife
- Tweezers, sterile
- FisherBrand Filtra Bags (Fisher Scientific Co., Pittsburgh, PA)
- Plastic Bucket to hold Filtra Bag
- 100ml graduated cylinder
- 100ml 0.1% Peptone dH_2O
- Seward Stomacher 400 Lab System (Seward Ltd., Thetford, Norfolk, England)
- 2.0ml Pipette Pump™ Pipettor #F37897 (Bel-Art Products, Pequannock, NJ)
- 1.0ml pipette Cat.#13-678-11B (Fisher Scientific Co., Pittsburgh, PA)
- 9ml 0.1% Peptone test tubes
- Test tube rack
- Vortex Genie 2, Cat#12-812 (Fisher Scientific Co., Pittsburgh, PA)
- Bunsen Burner, Model #1201-21 (Fisher Scientific Co.)
- Disposable Plastic Petri Dishes 10x15mm Cat#08-752-12 (Fisher Scientific Co.)
- Disposable anaerobic jar (Key Scientific Products, Round Rock, TX)
- Oxoid AnaeroGen™ AN35 sachet bag (Oxoid Ltd., Basingstoke, Hampshire, England)
- Oxoid Anaerobic Indicator BR55 (Oxoid Ltd.)
- Leica Quebec Darkfield Colony Counter Model #3325 (Wetzlar, Germany)
- Thumb counter

Safety Precautions:

- Gloves

Procedure:

1. Download all cooling data from Atkins #37313 data recording thermometer to laptop computer before analysis.
2. Place cooled turkey on sanitized cutting board.
3. Divide turkey using aseptic knife in approximate location of inoculum.
4. Carve out all green-dyed turkey meat using knife and sterile tweezers and place into one side of Filtra bag.
5. Macerate dyed meat by hand, until all large chunks are broken up.
6. Add 100ml 0.1% Peptone dH_2O and continue to macerate by hand, until dyed turkey meat is well pulverized.
7. Place Filtra bag in Stomacher and pulverize on normal speed for 60 seconds.
8. Remove Filtra bag from Stomacher
9. Using 2.0ml Pipette Pump™ Pipettor, 1.0ml pipette, and 9ml 0.1% Peptone test tubes, plate 1/10 dilutions of 0.1ml stomached liquid from 10^{-2} to 10^{-7} onto 10x15mm Disposable Plastic Petri Dishes.
10. Pour plate with SFP D-Cycloserine agar. [*see Procedure #4 – Preparation of Shahidi-Ferguson-*Perfringens* (SFP) agar base enriched with filter-sterilized D-Cycloserine solution].
11. Allow plates to harden quickly (it helps if you spread them out in a single layer on the counter).
12. Place hardened plates, upside-down, in an anaerobic jar.
13. Place an Oxoid Anaerobic Indicator BR55 on top of plates and an Oxoid AnaeroGen™ AN35 sachet bag inside the jar next to the inoculated plates and close jar quickly.
14. Incubate inoculated plates anaerobically at 37° for 24 hours.
15. Count $\log_{10}$ CFU/roast with Leica Quebec Darkfield Colony Counter, using thumb counter.
16. Record $\log_{10}$ CFU/roast counts of *Clostridium perfringens* in laboratory notebook.

APPENDIX B. RAW DATA

***C. perfringens* Counts (log_{10} CFU/roast) in Turkey, 3 Replications**

Treatment	Time from 57°C to 5°C In hrs:mins	Initial Spore Count of log_{10} CFU in roasts before cooking	Viable Counts of log_{10} CFU in roasts following cooling	Change in counts of log_{10} CFU in roasts following cooling
Quartered				
Replication 1	8:00	3.53	<100 cells	≥-3.53
Replication 2.1	9:40	4.74	3.08	-1.66
Replication 2.2	9:40	4.74	<100 cells	≥-4.74
Replication 3.1	8:10	4.41	2.6	-1.81
Replication 3.2	8:10	4.41	2.65	-1.76
$\bar{x}$	**8:44**	**4.37**	**≥1.67**	**≥-2.7**
±SD	:51	0.50	≤1.53	≤1.38
Blast Chiller				
Replication 1	12:30	3.53	3.54	0.01
Replication 2	14:00	4.74	3.28	-1.46
Replication 3	10:50	4.41	3.28	-1.13
$\bar{x}$	**12:26**	**4.23**	**3.37**	**-0.86**
±SD	1:35	0.63	0.15	0.77
Walk-In				
Replication 1	16:30	3.53	5.83	2.3
Replication 2	22:20	4.74	5.76	1.02
Replication 3	21:10	4.41	5.6	1.19
$\bar{x}$	**20:00**	**4.23**	**5.73**	**1.50**
±SD	3:05	0.63	0.12	0.70
Three-Bagged				
Replication 1	28:00	3.53	8.2	4.67
Replication 2	27:40	4.74	8.6	3.86
Replication 3	28:50	4.41	7.89	3.48
$\bar{x}$	**28:10**	**4.23**	**8.23**	**4.00**
±SD	0:36	0.63	0.36	0.61

Note: None of the cooling treatments met the amended 2001 FDA Food Code guidelines.

REFERENCES

Allen, R. L. (2000, Oct. 30). Study shows shrinking consumer confidence in food-safety practices. *Nation's Restaurant News,* pp. 1, 87.

Bryan, F. L. (1988). Risks of practices, procedures and processes that lead to outbreaks of foodborne diseases. *Journal of Food Protection, 51*, 663-673.

Doyle, E. (2002). Survival and growth of Clostridium perfringens during the cooling step of thermal processing of meat products. Madison, Wisconsin: University of Wisconsin, Food Research Institute.

Duncan, C. L., & Strong, D. H. (1968). Improved medium for sporulation of *Clostridium perfringens*. *Applied Microbiology, 16,* 82-89.

Food Safety and Inspection Service. (2002). Hot holding temperatures. Retrieved May 12, 2003, from http://www.fsis.usda.gov/OPHS/NACMCF/2002/rep_hothold1.htm

Food and Drug Administration. (2000). Report of the FDA Retail Food Program Database of Foodborne Illness Risk Factors. Available: http://vm.cfsan.fda.gov/~acrobat/retrsk.pdf. Accessed November 7, 2003.

FDA/CFSAN. (2003). Bad bug book: Introduction to foodborne pathogenic microorganisms. Retrieved September 29, 2003, from http://www.cfsan.fda.gov/~mow/intro.html

FDA/CFSAN FDA 2001 Food Code. (2001). Destruction of organisms - raw animal foods. 2001 Food Code. In *U. S. Department of Health and Human Services Public Health Service Food and Drug Administration 2001 Food Code* (Chap.

3). Retrieved September 10, 2003, from http://www.cfsan.fda.gov/~dms/fc01-3.html

FDA/CFSAN FDA Supplement to the 2001 Food Code (2003). Food Amendments, Additions, and Deletions. In *U. S. Department of Health and Human Services Public Health Service Food and Drug Administration Supplement to the 2001 Food Code* (Chap. 3). Retrieved May 29, 2004, from http://www.cfsan.fda.gov/~dms/fc01-sup.html#p1

Henroid, D., & Sneed, J. (2004). Readiness to implement hazard analysis critical control point (HACCP) systems in Iowa schools. *Journal of the American Dietetic Association, 104,* 180-185.

Heredia, N. L., & Labbé, R.G. (2001). Clostridium perfringens. In R.G. Labbé and S. Garcia (Eds.), *Guide to Foodborne Pathogens* (pp. 133-141). New York, New York: John Wiley & Sons, Inc.

Juneja, V. K., Snyder, O. P., & Cygnarowitz-Provost, M. (1994). Influence of cooling rate on outgrowth of *Clostridium perfringens* spores in cooked ground beef. *Journal of Food Protection, 57,* 1063-1067.

Juneja, V. K., Call, J. E., & Miller, A. J. (1993). Evaluation of methylxanthines and related compounds to enhance *Clostridium perfringens* sporulation using a modified Duncan and Strong medium. *Journal of Rapid Methods and Automation in Microbiology, 2,* 203-218.

McNab, W. B. (1998). A general framework illustrating an approach to quantitative microbial food safety risk assessment. *Journal of Food Protection, 61*, 1216-1228.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., et al. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases, 5*, 607-625.

National Food Service Management Institute. (1999). Chili con carne with beans. In USDA Recipes for Child Nutrition Programs. Available: http://www.nfsmi.org/Information/recipes/d_20.pdf. Accessed May 31, 2004.

National Restaurant Association. (2002a). Frequently asked questions. Retrieved September 24, 2003, from the World Wide Web: http://www.restaurant.org/faq.cfm#meals

National Restaurant Association. (2002b). Industry at a Glance. Retrieved September 25, 2003, from the World Wide Web: http://www.restaurant.org/research/ind_glance.cfm

National Restaurant Association Educational Foundation. (2004). *ServSafe® essentials (3rd Ed.)*. New York: John Wiley & Sons, Inc.

Owens, J. L. (Ed.). (1991). *ASM style manual (2nd ed.)*. Washington D.C.: American Society for Microbiology.

Ray, B. (2001). *Fundamental food microbiology (2nd ed.)*. Boca Raton: CRC Press.

Snyder, O. P., & Labalestra, J. A. (2004). Comparison of the bimetallic coil thermometer and thermocouple for validating food cooling. *Food Protection Trends, 24*, 423-428.

Snyder, O. P. (1997). Two-inch and four-inch food cooling in a commercial walk-in refrigerator. *Dairy, Food and Environmental Sanitation, 17*, 398-404.

Steele, F. M., & Wright, K. H. (2001). Cooling rate effect on outgrowth of *Clostridium perfringens* in cooked, ready-to-eat turkey breast roasts. *Poultry Science, 80, 813-816.*

U.S. Department of Agriculture, Food and Nutrition Service. (2003). Testimony of Eric M. Bost, Under Secretary, Food, Nutrition and Consumer Services before the Senate Committee on Agriculture, Nutrition and Forestry. Retrieved June 13, 2004, from the World Wide Web: http://www.fns.usda.gov/cga/Speeches/CT040303.html

U.S. Department of Agriculture, Food Safety and Inspection Service. (1999). Appendix B – compliance guidelines for cooling heat-treated meat and poultry products (stabilization). Retrieved June 12, 2004, from the World Wide Web: http://www.fsis.usda.gov/OA/fr/95033F-b.htm

U.S. General Accounting Office. (2003). School meal programs: Few outbreaks of foodborne illness reported, but opportunities exist to enhance outbreak data and food safety practices. Available: http://www.gao.gov/new.items/d03530.pdf. Accessed November 7, 2003.

U.S. General Accounting Office. (2002). Food safety: Continued vigilance needed to ensure safety of school meals. Available: http://www.gao.gov/new.items/d02669t.pdf. Accessed November 7, 2003.

ACKNOWLEDGMENTS

I would like to extend my sincere thanks and gratitude to my major professor, Dr. Jeannie Sneed, whose conscientious mentoring has inspired me to learn and grow both as a scholar and as an individual. I respect her commitment to ethics and academic excellence, her thorough knowledge of her field, and her patience as a truly effective mentor. In addition, I would like to extend sincere thanks to my committee members, Dr. Aubrey Mendonca for his expert microbiological support and enthusiasm and to Dr. Amit Sharma for his insight and guidance. Finally, I sincerely thank Bledar Bisha for all of his help and guidance in the microbiology laboratory.

I would like to express my gratitude to my instructors, my employers, and my colleagues that I have known throughout my 20-year career in the foodservice industry. They helped shape my interest in the restaurant industry, which eventually instilled in me the desire to explore graduate studies. I will pass the knowledge I have gained from my experience to others as it was taught to me, with patience and professionalism.

Special recognition should be given to: (a) the USDA for their in-kind contribution of turkey roasts; (b) Mrs. Beth Hanna, West Des Moines, IA School Foodservice for the in-kind contribution of ground beef; (c) Dr. Sam Beattie for providing recording thermometers; and (d) the ISU College of Family and Consumer Sciences for providing the financial support for my thesis.